Photoreceptor metabolism and visually guided landing behaviour of flies

Tinbergen, Jan

PHOTORECEPTOR METABOLISM AND VISUALLY GUIDED LANDING BEHAVIOUR OF FLIES

J TINBERGEN

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Stellingen.

1 De door Reichardt ontwikkelde modellen voor bewegingszien, die vooral getest zijn voor optomotorische torsie-reacties van vliegende vliegen, vormen een goed uitgangspunt voor het begrijpen van de bewegingsgevoeligheid die optreedt bij de landingsreactie.

Reichardt, W. (1987) J. Comp. Physiol. *161*: 533-547.

2 De bij de landingsreactie van de vlieg optredende verschillen in gevoeligheid voor bewegende gepolariseerde patronen kunnen verklaard worden uit de specifieke lamina-receptor koppelingen in het equatoriale deel van het samengestelde oog.

Eckert, H. (1983) Naturwissenschaften *70*: 150-151.

3 De door Borst en Bahde voor de landingsreactie voorgestelde latentietijden van ca. 25 ms zijn in de praktijk niet mogelijk vanwege de 25 - 35 ms durende delay, die optreedt tussen de visuele stimulus en het uitsteken van de poten.

Borst, A., Bahde, S. (1987) Biol. Cybern. *56*: 217-224.

4 Aggressief gedrag tussen voedselzoekende vleesvliegen is seizoensafhankelijk en waarschijnlijk gekoppeld aan het voorkomen van predatoren.

5 De huidige televisietoestellen en personal computers werken met een beeldopbouw die marginaal is vergeleken met het spatïele en temporele oplossend vermogen van het eraan blootgestelde visueel systeem.

6 Uit nader onderzoek zal moeten blijken of stimulatie van het evenwichtsorgaan met geluid leidt tot een sensatie, die bij totale doofheid een alternatief kan vormen voor b.v. het toepassen van een cochleair geïmplanteerde electrode.

7 Het gesubsidieerd verspreiden van mestoverschotten stinkt.

8 Het aantal verkeersslachtoffers onder de door kunstlicht aangetrokken en erdoor verblinde nachtvlinders is verontrustend.

9 Voor de bescherming van onze vogels is in toenemende mate een internationale aanpak noodzakelijk.

J.Tinbergen
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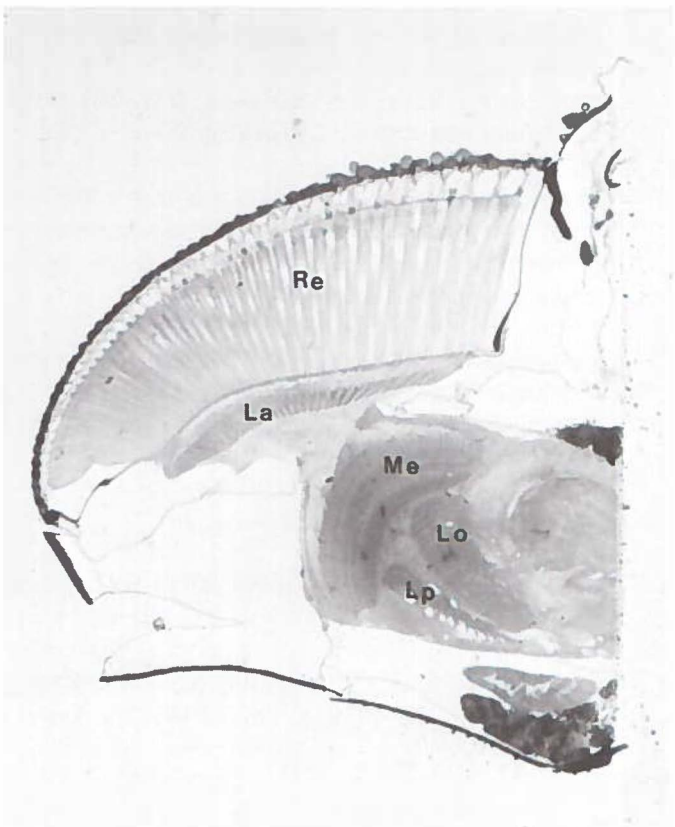

Fig. 1. Horizontal cross-section of the compound eye and optic lobe of the blowfly *Calliphora erythrocephala.* Re: retina, La: lamina, Me: medulla, Lo: lobula, Lp: lobula plate.

Chapter I

INTRODUCTION

Vision primarily depends on the absorption of light by the specialized visual pigment molecules, located in the visual sense cells. This light absorption induces a change in the conformation of the visual pigment molecules, which in turn triggers a cascade of amplification steps. This process finally results in a change in the membrane conductance and electric potential of the visual sense cell. The change in membrane potential is called the receptor potential. The visual sense cells, in the retina, are part of the eye, in which accessory light distributing and light screening optical structures together determine the directional sensitivity, i.e., a visual cell receives predominantly light from one direction in space. The activity of the sense cells together thus build an image of the environment.

The receptor potentials of the sense cells are transmitted to, and processed by, the neuronal network of the visual system. This visual processing, in various ways and at different levels finally results in specific motor commands and muscular action by which the animal responds to the distribution of light in the environment, and specifically to the changes therein.

Flying insects have a surprisingly high developed visual system, capable to perform many different tasks with high accuracy. For instance, motion detection, polarized light detection, colour vision and pattern recognition are well-established.

The compound eyes and optic lobes of the fly's visual system have a structural complexity, which is intermediate to that of higher vertebrates and lower organisms. An outstanding feature is that they are built up very systematically and regularly. Many different aspects of the structure, general principles and specific characteristics of visual processes have already been investigated and unravelled (see for reviews Autrum 1979,1981a,b; Ali 1984; Hardie 1985).

In the present thesis optical methods have been applied to investigate *in vivo* the dynamics of oxidative metabolic processes in the cell bodies of the visual sense cells. Fluorescence measurements of the eyes of white eyed mutant flies, lacking retinal screening pigments, yielded rapid changes in the redox state of mitochondrial enzymes, related with the light-dependent need for metabolic energy of the sense cells (Chapters II-IV).

In behavioural experiments on the landing response the directional selective movement sensitivity of flies has been studied in tethered, stationary flying flies (Chapters V-VII).

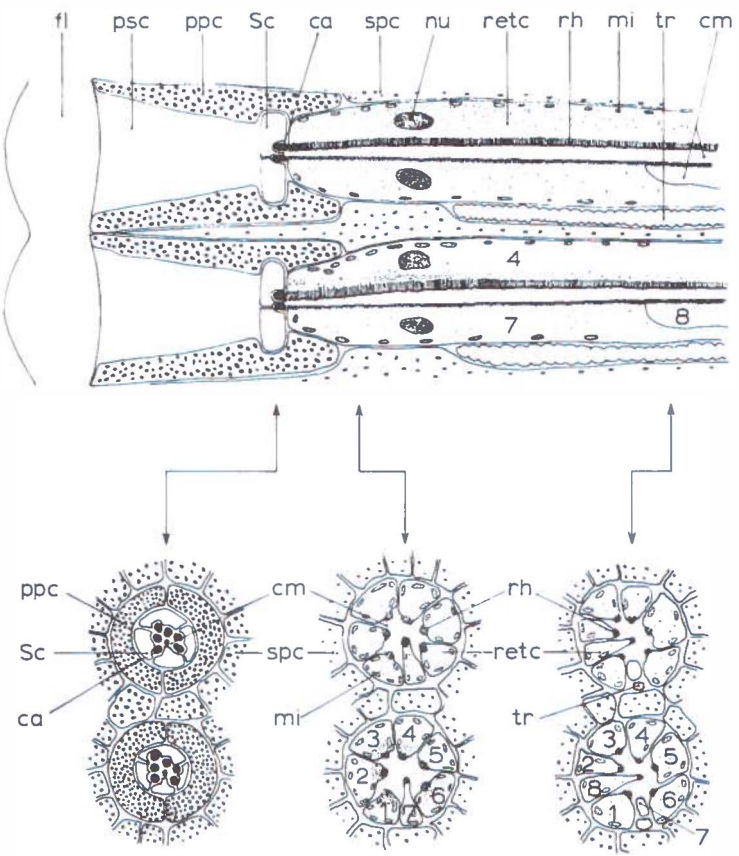

Fig. 2. Two ommatidia from a fly retina. A fly ommatidium consists of a facet lens (fl), a pseudocone (psc), four Semper cells (Sc), eight retinula or sense cells (retc), numbered R1 to R8, two primary pigment cells (ppc), six secondary pigment cells (sps), and a tracheolus (tr). The rhabdomeres have a microvillar structure. Distally from the rhabdomeres the caps (ca) are situated. The rhabdomeres are separated by the ommatidial cavity or central matrix (cm). Inside the photoreceptor cells nucleus (nu) and mitochondria (m) are shown. The upper ommatidium is dark adapted, the lower ommatidium shows the light adapted state. (From Stavenga 1974)

The structure of the compound eye of the fly and the optical lobe of the brain is described briefly below; for more details see Hardie (1985), Strausfeld (1976,1984), Strausfeld and Nässel (1981) and Hausen (1984).

Fig. 1 represents a horizontal cross-section of the lateral part of the head, showing the retina and the four separate ganglia of the optic lobe, the lamina ganglionaris, medulla, lobula and lobula plate. The functional unit of the compound eye, the ommatidium, is marked by the transparant facet lens at its distal end (Fig. 2). It contains a small retinula with 8 sense cells. Each sense cells possesses a rhabdomere, consisting of densely packed microvilli in which the 10^8 visual pigment molecules (Hamdorf 1979) are embedded in the plasma membrane. The distal end of the rhabdomeres is covered by a cap, which is located in the focal plane of the facet lens. Light entering through the lens and arriving at the distal end of the rhabdomere, is propagated in the rhabdomere due to the high refractive index of the medium within the rhabdomere boundary. Thus light can be effectively absorbed by the visual pigment molecules. There are six main cells called R1-6 with long rhabdomeres containing the same type of visual pigment. The rhabdomeres of cells R7 and R8 are shorter and form a tandem. They generally contain visual pigments with spectral properties different from that of cells R1-6. The rhabdomeres are separated by a clear central matrix; the eye is of the so-called open rhabdom type. In the cell bodies of the sense cells mitochondria are abundant; they are predominantly located near the cell membrane. The tracheoli, supplying the necessary oxygen, penetrate the major part of the retina. The ommatidia are separated by pigment cells, containing light absorbing screening pigment granules which block light coming from the side. Within the sense cells tiny pigment granules are found, which migrate towards the rhabdomere at higher light levels and therefore together act as a pupil.

Stavenga (1974), Beersma (1979), Smakman (1985) and van Hateren (1987) have investigated receptor optics and related visual processes in the compound eye.

In the optic lobe (see Strausfeld 1976,1984; Strausfeld and Nässel 1981; Hausen 1984) roughly 10^6 neurons are located. The first layer, the lamina, consists of an array of identical clusters of neurons, so-called neurocartridges, one below each ommatidium in a retinotopic organisation. In each cartridge six R1-6 receptors terminate and have synaptic contacts with second order neurons, monopolar cells. The 6 cells which terminate together are those receiving light from the same direction in space. The monopolar cells have interactions within the neurocartridges and, via the first optic chiasma, with several third order cells at different levels of the medulla. In the medulla the retinotopic organization of the neuropile is still

present. The sense cell axons R7 and R8 terminate in the medulla and have synaptic contacts to several cell types at different levels of the medulla. A second optic chiasma links the medulla to the lobula-complex.

Especially structure and characteristics of large field tangential neurons in the lobula plate have been extensively investigated (Strausfeld 1976,1984; Hausen 1984). The different types of large field tangential neurons have synaptic connections with elements corresponding to large parts of the visual field, and in some cases also with the contralateral visual field. These neurons are predominantly movement sensitive with activation by movement in the preferred direction of movement and inhibition by movement in the opposite direction (Mastebroek 1974; Zaagman 1977; Lenting 1985; de Ruyter van Steveninck 1986). Those cells which are sensitive to horizontal movement have their connections at a deeper level of the lobula plate than those sensitive to vertical movement. The neurons in the cervical connective connect the optical lobe with the thoracic ganglia. The latter ganglia innervate the motor command system, governing wing and leg movements, and therefore the landing behaviour.

The transduction mechanism of all sense cells in the fly's compound eye is probably in principle identical, except for a difference in gain between R1-6 and R7 and R8. Upon illumination the visual sense cells respond with a graded, intensity dependent depolarization of the plasma membrane, e.g. Leutscher-Hazelhoff (1973) and Muijser (1980). The spectral properties of the sense cells, which are primarily determined by the absorption spectrum of the visual pigment, are identical in the main type of sense cells, R1-6. These cells have a UV-green double-peaked spectral sensitivity. It has been recently proved that the visual pigment is 3-hydroxyretinal-based and therefore the nomenclature of the visual pigment, rhodopsin, is proposed to be xanthopsin (Vogt 1983). This visual pigment alone can not explain the double peaked spectral sensitivity. The UV-peak is predominantly due to a sensitizing pigment, probably 3-hydroxyretinol, which upon UV-illumination transfers energy to the visual pigment. In cells R7 and R8 different spectral sensitivities have been found. The main classes are two types of UV-sensitive R7 cells and blue-sensitive and yellow-sensitive R8 cells (see Hardie 1985).

The various cell types have been investigated *in vivo* with optical methods, in which the autofluorescence of the visual pigments and photostable pigments identified the different types of sense cells (Franceschini et al. 1981). The red autofluorescence of the visual pigment in the rhabdomeres of the R1-6 cells originates from meta states of the photoconvertible pigment (Stavenga et al. 1984).

The fluorescence studies presented in this thesis have been mainly concentrated on the autofluorescence emerging from the cell bodies of the

sense cells. The dynamic change in the emission intensity discovered in white eyed mutant blowflies is described in Chapter II. The emission change is not measurable from wildtype flies, because the presence of the screening pigments and the dynamics of the pupil mechanism obstruct and complicate the signal. The characteristics of the UV-induced blue emission and the blue-induced green emission of the eye have been investigated in Chapter III. In this chapter, furthermore, the transmission of the complete eye has been studied under normal and hypoxia conditions. From the fluorescence and transmission studies it has been concluded that mitochondrial activity can be studied in the intact fly via the pigments of the respiratory chain (see Lehninger 1970). The dynamic change in the green emission reflects a transient incrsystemease in flavoprotein fluorescence, or, a transient increase in metabolic activity. This effect is discussed in relation with the phototransduction process and is described in Chapter IV. This research has yielded that the oxidative metabolic processes in the cell bodies of the sense cells are modified by illumination and that these effects are reflected in the emission changes. Presumably, the demand for metabolic energy in the sense cells increases upon illumination due to a higher rate of ion exchange and pump activity.

In the behavioural studies on the landing response of stationary flying flies, presented in Chapter V, a description of the response pattern is given, and has been concentrated on the responsivity to various approaching and non-approaching movement stimuli. A more detailed analysis of the velocity dependence of the sensitivity to horizontal movement stimuli, as is reflected in the amount of movement necessary to elicit the response, has been presented in Chapter VI. In the last chapter, bichromatic patterns have been used to test the dependence of the landing response on the spectral characteristics of the movement stimulus (Chapter VII).

References.

Ali , M.A. (1984) Photoreception and vision in invertebrates. Plenum Press, New York and London.

Autrum, H. (1979,1981a,b) Handbook of sensory physiology, vol VII/6A,B,C. Springer, Berlin Heidelberg New York.

Beersma, D.G.M. (1979) Spatial characteristics of the visual field of flies. Thesis Groningen.

Franceschini, N., Kirschfeld, K., Minke, B. (1981) Fluorescence of photoreceptor cells observed in vivi. Science 213:1264-1267

Hamdorf, K. (1979) The physiology of invertebrate visual pigment. In: Autrum, H. (ed) Handbook of sensory physiology VII/6A. Springer,

Berlin Heidelberg New York, pp 145-224
Hardie, R.C. (1985) Functional organization of the fly retina. In: Ottoson, D. (ed.) Progress in sensory physiology 5. Springer, Berlin Heidelberg New York, pp 1-79
Hateren, van, H. (1987) Photoreceptor optics and neural microcircuitry in the insect eye. Thesis Groningen.
Hausen, K. (1984) The lobula complex of the fly: Structure, function and significance in visual behaviour. In: Ali, M.A.(ed) Photoreception and vision in invertebrates. Plenum Press, New York and London, pp 523-559
Lehninger, A.L. (1970) Biochemistry. Worth, New York.
Lenting, B.P.M. (1985) Functional characteristics of a wide-field movement processing neuron in the blowfly visual system. Thesis Groningen.
Leutscher-Hazelhoff, J.T. (1973) Photoreceptor performance in the blowfly. Thesis Groningen.
Mastebroek, H.A.K. (1974) Stochastic structure of neural activity in the visual system of the blowfly. Thesis Groningen.
Muijser, H. (1980) Investigations into the phototransduction in fly visual sense cells. Thesis Groningen.
Smakman, J.G.J. (1985) Angular and spectral sensitivity of blowfly photoreceptors. Thesis Groningen.
Stavenga, D.G. (1974) Visual receptor optics, rhodopsin and pupil in fly retinula cells. Thesis Groningen.
Stavenga, D.G., Franceschini, N., Kirschfeld, K (1984) Fluorescence of housefly visual pigment. Photochemistry and Photobiology 40: 653-659
Strausfeld, N.J. (1976) Atlas of an insect brain. Springer, Berlin Heidelberg New York.
Strausfeld, N.J., Nässel, D.R. (1981) Neuroarchitectures serving compound eyes of crustacea and insects. In: Autrum, H. (ed) Handbook of sensory physiology, vol VII/6B. Springer, Berlin Heidelberg New York,pp 1-132
Vogt, K. (1983) Is the fly visual pigment a rhodopsin? Z. Naturforsch. 38c: 329-333
Zaagman, W.H. (1977) Some characteristics of the neural activity of directionally selective movement detectors in the visual system of the blowfly. Thesis Groningen.

Chapter II

LIGHT DEPENDENCE OF OXIDATIVE METABOLISM IN FLY COMPOUND EYES STUDIED IN VIVO BY MICROSPECTROFLUOROMETRY

Arthropod photoreceptors depend on oxidative metabolism for maintenance of the resting potential (horseshoe crab [1], bee [1], fruitfly [2]) and sensitivity to light (owlfly [3], locust [4]). In respiration measurements on isolated retinae (bee [5, 6], blowfly [5, 7, 8] it was demonstrated that intense illumination distinctly elevates the rate of oxygen consumption by the photoreceptor cells. Here we report microspectrofluorometric measurements on light-induced oxidative metabolic processes in the compound eye of completely intact, living blowflies.

Fluorescence was measured from approximately 300 ommatidia of a compound eye of the blowfly *Calliphora erythrocephala* with a Leitz Orthoplan microscope equipped with a Leitz NPL 10 objective (aperture 0.20), a Ploemopak illuminator and a Compact photometer system. Completely intact and living animals could be investigated by selecting the white-eyed mutant chalky. This mutant lacks the retinal screening pigments, which in wild-type flies obstructs *in vivo* fluorometry, but otherwise the retinal properties of the mutant appear to be identical to those of the wild type [9-13].

The main visual pigment of blowflies is a rhodopsin absorbing maximally in the blue-green (λ_{max}=495 nm), which after light absorption converts into a thermostable metarhodopsin, absorbing maximally in the orange (λ_{max}=580 nm) [10-14]. Recently it was established that the metarhodopsin state upon orange excitation distinctly fluoresces in the far-red [13-15].

Applying blue excitation light and measuring in the green we failed to see any indication for rhodopsin fluorescence, but found that blue light, when delivered to the eye after a few seconds of darkness, induces a transient increase in green fluorescence. After longer dark times a biphasic process with a maximum at 1-2 s after light onset emerges (Fig. 1). Because the amplitude appears to be governed by a mechanism having a time constant in the order of 15-30 s we hypothesized that the system is related to recovery of the photoreceptors' light sensitivity in the dark. The main phase of this so-called dark-adaptation process occurs within that same span of time [11-16].

Fly photoreceptors respond to illumination by a depolarization of the cell

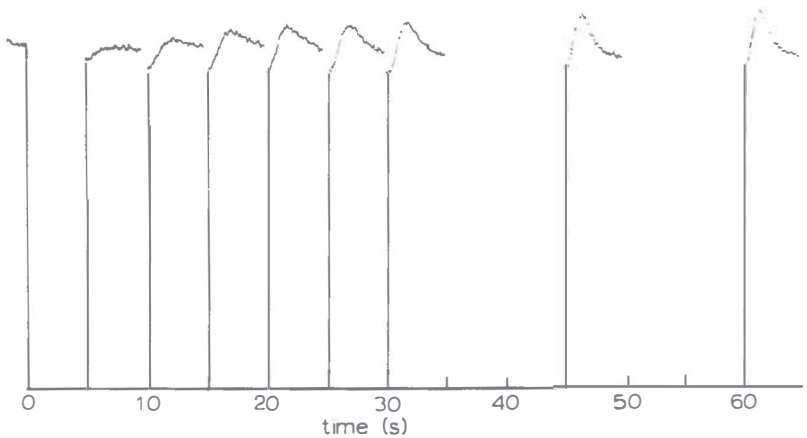

Fig. 1. Autofluorescence measured from the eye of a completely intact, living blowfly *Calliphora erythrocephala* mutant chalky. The excitation light, wavelength 456 nm and intensity 1.2×10^{17} quanta cm^{-2} s^{-1}, was applied during 5 s. Emission,measured in the wavelength range 510-600 nm, exhibits a biphasic time course, the shape of which depends on the dark time The response is presented with time of onset at the preceding dark time (abscissa).

membrane. Since the light sensitivity is reduced by a lowered oxygen tension [1-4] we applied a stream of nitrogen to the fly and measured its effect on the photoreceptors' fluorescence. Fig. 2 shows the effect of hypoxia during interrupted and continuous light, respectively. After a few seconds of depletion of oxygen the fluorescence signal falls and, furthermore, the light induced dynamic process vanishes. On the other hand, recovery from hypoxia is almost instantaneous and appears to induce a strong increase in both the static and the dynamic fluorescence signal. (Measurements performed during application of pure oxygen yielded identical results to those obtained in air.) The dynamics of the fluorescence signal depends, furthermore, distinctly on temperature. At low temperatures the process slows down, and, ultimately, no fluorescence change is observable anymore at $\leq 5°$.

A preliminary analysis of the eye's blue-induced emission spectrum

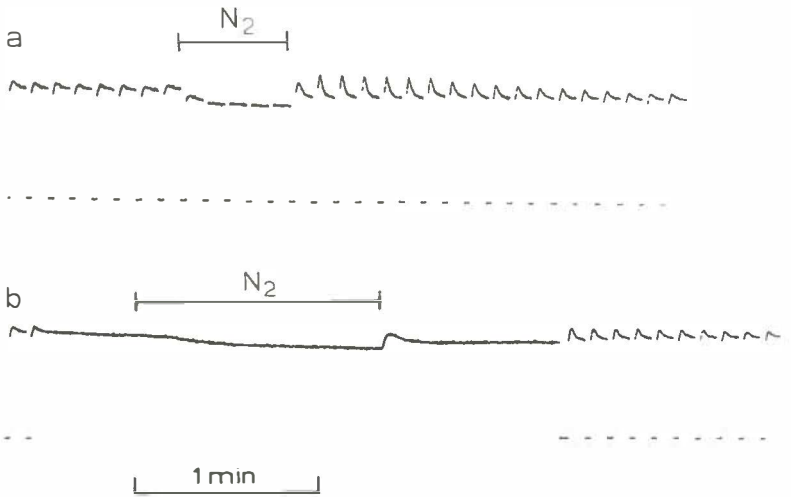

Fig. 2. Hypoxia severely affects the fly's eye autofluorescence. The conditions were the same as in Fig. 1, except that the 5 s flashes were interrupted by a 30 s dark time (during which the recorder halted). Application of nitrogen rapidly lowers the fluorescence level, and the biphasic curve is flattened; after hypoxia an enhanced light-induced fluorescence change occurs which gradually settles down (a). Essentially the same effects are seen during continuous light (b).

shows a broad green peak around 520-530 nm (see also [15]), the height of which is lowered by anoxia.

These findings can directly related to the studies of Chance et al. [17-19] on the flavins of the mitochondrial respiratory chain. They showed that anoxia of mitochondria results in a decrease of oxidized flavin compounds and thus in a fall in blue-induced green emission, and vise versa, that an increased rate of oxygen consumption is accompanied by an emission increase. Hence we suggest that the fluorescence changes induced by the illumination of fly photoreceptors are caused by enhanced oxidative metabolic processes, also because the rapid dynamics of the green fluorescence, being in the order of a few seconds, accord with the measurements of oxygen consumption induced by light flashes in isolated retinae [20,21].

This view is supported by our measurements of ultraviolet induced blue emission. The blowfly eye then exhibits a blue fluorescence peaking at about

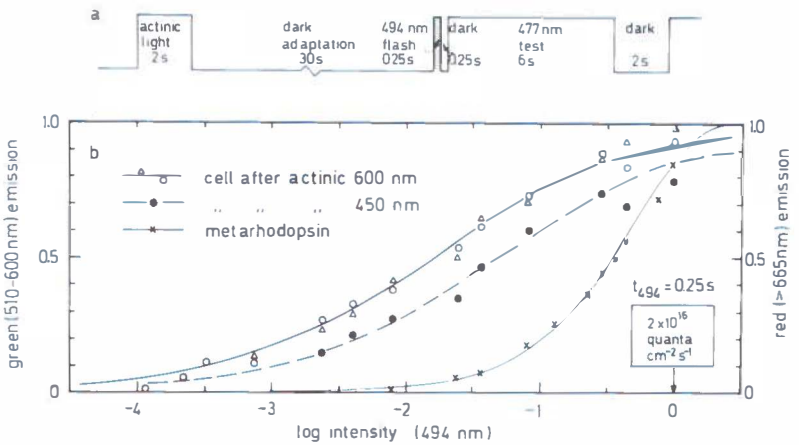

Fig. 3. Fluorescence changes in blowfly eyes caused by 494 nm flashes (a). Previous to the flash a 2 s actinic light of either ≈600 nm (Balzers K 60) or ≈450 nm (Balzers K 45) and a 30 s dark adaptation time was given. After the 0.25 s lasting flash a 477 nm test light (9.1×10^{15} quanta cm^{-2} s^{-1}) was applied 0.25 s later. The induced green emission (510-600 nm) was measured and in (b) the difference of the initial value from the value obtained after a low-intensity flash is drawn normalized to the maximum increment. △, o : data from two runs on experiments performed from low to high intensities after actinic 600 nm. Hysteresis effects were negligible showing a complete reset by the actinic light and the following dark time to the same cell condition. The data are approximated by the hyperbolic function [22, 23] $(RI^n)/((RI)^n+1)$}, with n=0.6. I is the intensity of the 494 nm light; R^{-1}, the intensity where the fluorescence change is half-maximum, is 0.32×10^{15} quanta cm^{-2} s^{-1} and 10^{15} quanta cm^{-2} s^{-1} after 600 nm and 450 nm preadaptation, respectively. The range shift is explained from the difference in rhodopsin content being ≈100% and ≈30%, respectively. The red fluorescence of metarhodopsin created by 494 nm flashes of various intensity, when starting from an initial 100% rhodopsin situation, is measured >665 nm (613 nm excitation). The resulting emission values are approximated by $1-\exp(-0.69IR)$ with $R^{-1} = 7.3 \times 10^{15}$ quanta cm^{-2} s^{-1}.

460 nm [9, 15]. Upon illumination the ultraviolet-induced blue emission drops with the same dynamics as that of the green emission increase seen in the blue induced fluorescence experiments (the relative change is smaller however).

Since oxidation of NADH results in a loss of blue fluorescence [18] we conclude that illumination of fly photoreceptors causes an increased oxidized state of the molecules in the mitochondrial respiratory chain.

The rapid metabolic processes manifest themselves at bright light intensities only. In the experiment of Fig. 3 the intensity dependence was investigated along the following procedure. In sequence, a 2 s lasting actinic light, a 30 s dark adaptation time and a 0.25 s flash of 494 nm (at the wavelength of the rhodopsin absorption peak) was applied. The effect of the flash was tested by a blue (477 nm) light given 0.25 s later and the initial value of of the blue-induced green (510-600 nm) emission was registered. We executed two series of experiments; in the first series the actinic light was orange (Balzers K 60) and in the second blue (Balzers K 45), in order to establish a high (≈100%) and a low (≈30%) rhodopsin concentration, respectively, in the photoreceptors [10-12, 20]. It appears that the initial green fluorescence depends on the intensity of the preceding 494 nm flash by a hyperbolic function [22,23] (see legends of Fig. 3). A similar intensity dependence was previously found for the oxygen consumption by the isolated retina [7,8].

Several lines of evidence confirm the assumption that rhodopsin conversion triggers the enhanced metabolic activity. Firstly, preliminary measurements of the action spectrum indicate a clear correspondence of the oxygen consumption [8] and the spectral sensitivity of the main class of fly photoreceptors measured electrophysiologically [24]. Furthermore, the high- and low-rhodopsin curves (Fig. 3) are separated by 0.5 log unit, which can be explained from the difference in rhodopsin content. As the intensity-dependence curves in the two series are normalized we have to note that the saturating response is distinctly smaller in magnitude with low rhodopsin than with high rhodopsin, indicating a maintained demand for metabolic energy when metarhodopsin is abundant.

It is of obvious interest to relate light-induced mitochondrial activity with phototransduction processes in general. Because the principal step in phototransduction is rhodopsin conversion into metarhodopsin [11,24], we estimated the degree of rhodopsin conversion caused by the 494 nm flashes by measuring the resulting metarhodopsin fluorescence (613 nm excitation, >665 nm emission; for procedure see [13, 15]. As indicated in Fig. 3 the brightest flash applied was just insufficient to establish within the 0.25 s flash time the photosteady state. When we consider that of the 1-2 x 10^8 visual pigment molecules of fly photoreceptors [11, 12, 25] ≈60% exist in

the metarhodopsin state after prolonged 494 nm light [11-12] we can read from Fig. 3 that mitochondrial activity is half-maximum when in the order of 10^6 rhodopsin molecules are converted. On the other hand electrophysiological experiments indicate that the receptor potential from a dark adapted cell is half-maximum when about 10^4 rhodopsin molecules are converted, i.e. at an intensity where activation of the mitochondrial process becomes slightly noticeable ($\log I \approx -4$ in Fig. 3).

Since rhodopsin conversion results in an increased membrane permeability and thus in ionic currents, ionic pumps are activated [26]. The sodium pump is an ATP-ase and hence oxygen consumption is enhanced to recycle the depleted ATP [20-21, 26, 27]. In bee photoreceptors at least half, and probably more, of the energy produced by respiration is needed for the sodium pump [27, 28]. However, several other processes following rhodopsin to metarhodopsin conversion but preceding the ionic change draw on the supply of ATP as has been demonstrated recently for vertebrate rods (e.g. [29-31]) and *Limulus* ventral photoreceptors [32].

The recognition of the origin of the light induced fluorescence changes opens the possibility for non-invasive measurements on photoreceptor metabolism, an essential part of the phototransduction machinery.

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References

1.Bauman, F., Mauro, A.: Nature New Biol. *244*, 146 (1973)
2.Wong, F., et al.: Nature *264*, 661 (1976)
3.Hamdorf, K., Gogala, M., Stusek, P.: Biol. vestn. (Ljubljana) *26*, 107 (1978)
4.Payne, R.: J. Comp. Physiol. *142*, 181 (1981)
5.Autrum, H., Tscharntke, H.: Z. vergl. Physiol. *45*, 695 (1962)
6.Autrum, H., Hamdorf, K.: Z. vergl. Physiol. *48*, 266 (1964)
7.Hamdorf, K., Kaschef, A.H.: Z. vergl. Phyisol. *48*, 251 (1964)
8.Hamdorf, K., Langer, H.: Z. vergl. Physiol. *52*, 386 (1966)
9.Franceschini, N., Kirschfeld, K., Minke, B.: Science *231*, 1264 (1981)
10.Stavenga, D.G.: J. Comp. Physiol. *111*, 137 (1976)
11.Hamdorf, K., in: Handbook of Sensory Physiology, Vol. VII/6A, p. 145 (ed. Autrum, H.). Berlin: Springer 1979
12.Schwemer, J.: Habilitationsschrift Bochum 1979
13.Stavenga, D.G.: Biophys. Struct. Mech. *9*, 309 (1983)
14.Stavenga, D.G., Franceschini, N.: Invest. Ophthalmol. Vis. Sci. (Suppl.)

20, 111 (1981)
15.Stavenga, D.G., Franceschini, N., Kirschfeld, K.: Photochem. Photobiol. *40*, 653 (1984)
16.Laughlin, S.B., Hardie, R.C.: J. Comp. Physiol. *128*, 319 (1978)
17.Chance, B., Schoener, B., in: Flavins and Flavoproteins, p. 510 (ed. Slater, E.C.). New York: Academic Press 1966
18.Scholz, R., et al.: J. Biol. Chem. *244*, 2317 (1969)
19.Chance, B., et al., in: Flavins and Flavoproteins, p. 669 (ed. Kamin, H.). Baltimore: Univ. Park Press 1971
20.Hamdorf, K., Schwemer, J., in: Photoreceptor Optics, p. 263 (eds. Snyder, A.W., Menzel, R.). Berlin: Springer 1975
21.Tsacopoulos, M., Poitry, S.: J. Gen. Physiol. *80*, 19 (1982)
22.Laughlin, S.B., in: Handbook of Sensory Physiology, Vol. VII/6B, p. 133 (ed. Autrum, H.). Berlin: Springer 1981
23.Matic, T., Laughlin, S.B.: J. Comp. Physiol. *145*, 169 (1981)
24.Hardie, R.C.: J. Comp. Physiol. *129*, 19 (1979)
25.Hamdorf, K., Kirschfeld, K.: Nature *283*, 859 (1980)
26.Fain, G.L., Lisman, J.E.: Progr. Biophys. Mol. Biol. *37*, 91 (1981)
27.Coles, J.A., Tsacopoulos, M.: J. Exp. Biol. *95*, 75 (1981)
28.Tsacopoulos, M., et al.: Nature *301*, 604 (1983)
29.Liebman, P.A., Pugh, E.N.: Nature *287*, 734 (1980)
30.Wilden, U., Kuehn, H.: Biochemistry *21*, 3014 (1982)
31.Zuckerman, R., Schmidt, G.J., Dacko, S.M.: Proc. Nat. Acad. Sci. USA *79*, 6414 (1982)
32.Stern, J.E., Lisman, J.E.: Proc. Nat. Acad. Sci. USA *79*, 7580 (1982)

Chapter III

PHOTORECEPTOR REDOX STATE MONITORED *IN VIVO* BY TRANSMISSION AND FLUORESCENCE MICROSPECTROPHOTOMETRY IN BLOWFLY COMPOUND EYES

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Abstract—The transmission and fluorescence of the compound eye of living, intact blowflies *Calliphora erythrocephala*, mutant *chalky*, were studied microspectrophotometrically. Transmission spectra were recorded under four conditions. The fly was either in the normal air environment or in a nitrogen atmosphere, and in both cases the investigated eye was adapted to red and blue light, respectively.

The absorbance difference spectra obtained from the two chromatic adapted conditions showed the clear characteristics of the main visual pigment; the difference spectra for the air and the N_2 case were virtually identical.

The absorbance difference spectrum obtained from the air vs N_2 case was very similar to the redox difference spectrum of the pigments in the mitochondrial chain. The redox difference spectra obtained for the two photosteady states were essentially the same.

The fluorescence emission spectra induced by UV and blue excitation were measured with the fly in air and in a nitrogen atmosphere, respectively. The UV-induced blue emission increased under hypoxia, whereas the blue-induced green emission dropped. The changes are typical for a reduction of mitochondrial NADH and flavoproteins, respectively.

The transmission and fluorescence measurements corroborate each other and demonstrate mitochondrial activity in photoreceptors *in vivo* and non-invasively.

Photoreceptor metabolism Blowfly Hypoxia Microspectrophotometry Microspectrofluorometry Visual Pigments Mitochondrial pigments

INTRODUCTION

Microspectrophotometry of photoreceptor cells has become an almost routine technique in vision research since the first studies on visual pigments *in situ* were started a quarter of a century ago (rev. Liebman, 1972). Probably due to their primary role in vision the visual pigments have received the major attention of the spectroscopists. Later on, the photostable (e.g. screening) pigments, such as those in oil droplets have attracted extensive interest (e.g. Muntz, 1972). On the other hand, the pigments of the mitochondrial respiratory chain, the third pigment class of importance for photoreceptors, have gained less interest.

Liebman (1969) presented a record from the ellipsoid, i.e. the mitochondrial region, of a cone cell in *Necturus*. The record shows the presence of several types of cytochrome oxidative enzymes in the reduced state. Liebman stated: "It is not known if a metabolic response is initiated in the mitochondria by light absorbed in the photoreceptor outer segment nor is it apparent what time course such a response would take. The answer to these questions might be provided by microphotometry." Indeed, recently we observed (Stavenga and Tinbergen, 1983), in the course of our *in vivo* microspectrofluorometrical studies on the visual pigments in blowfly eyes, transient fluorescence changes, with a time course in the order of seconds, and hypothesized that these changes originated in light-induced redox changes in the mitochondrial respiratory chain. The evidence was mainly obtained from measurements on the dependence of the phenomenon on light intensity and oxygenation. We therefore decided to characterize the spectral properties of the pigments involved by both transmission and fluorescence microspectrophotometry.

MATERIALS AND METHODS

The blowfly *Calliphora erythrocephala*, white eyed mutant *chalky*, was investigated. This mu-

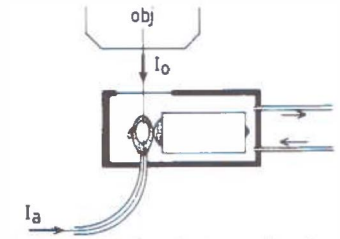

Fig. 1. Diagram of housing with immobilized intact fly. Arrows indicate gas flow for application of air or N_2 atmosphere. In the transmission measurements illumination I_a was applied via a small light guide at the ventral side of the eye. In the fluorescence measurements illumination I_o was applied via the objective of the microspectrophotometer.

tant lacks the screening pigments, which exist normally, i.e. in the wild type fly, in both the pigment cells and the photoreceptor cells, but in all other aspects, the retinal properties of mutant and wild type are considered identical.

The fly was immobilized with wax (and remained thus intact and alive; when occasionally a drop of sugar water is supplied, experiments with one and the same fly then can easily be extended over several days). The fly was subsequently mounted in a housing on a Leitz Universal stage. A window, consisting of a sealed-in cover slip, allowed the optical measurements (see Fig. 1). The composition of the atmosphere in the housing was changed through tubes connected to a selected gas cylinder. The atmospheres were always at room temperature.

For the transmission measurements a plastic light guide (diameter 0.5 mm) was introduced through a tightly fitting hole in the wall of the housing and its tip was positioned ventrally to the compound eye (Fig. 1). The other end received light from a 450 W Xe-arc filtered spectrally by an Oriel grating monochromomator. The objective of the microspectrophotometer (MSP) sampled light from *c*.

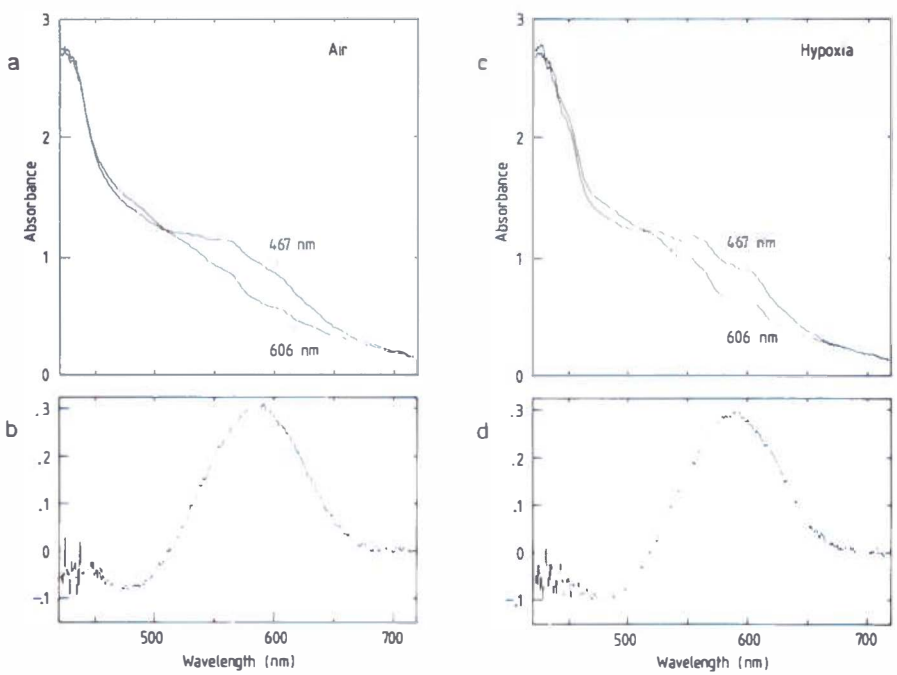

Fig. 2. Absorbance spectra of the photoreceptor layer of the blowfly eye and their absorbance difference spectra. (a) Absorbance spectra of the eye adapted to blue (467 nm) and red (606 nm) light, respectively, when placed in a normal (air) atmosphere. (b) Absorbance difference between the two spectra of *a*. (c) Absorbance spectra of the eye adapted to blue (467 nm) and red (606 nm) light under hypoxia conditions induced by a N_2 atmosphere. (d) Absorbance difference between the spectra of *c*. Absorbance was calculated as the $\log_{10}$ of the ratio between the light signal obtained from the light guide directly and that with the blowfly eye in the light beam. However, the ordinate values were lowered by approximately 3 log units for clarity's sake.

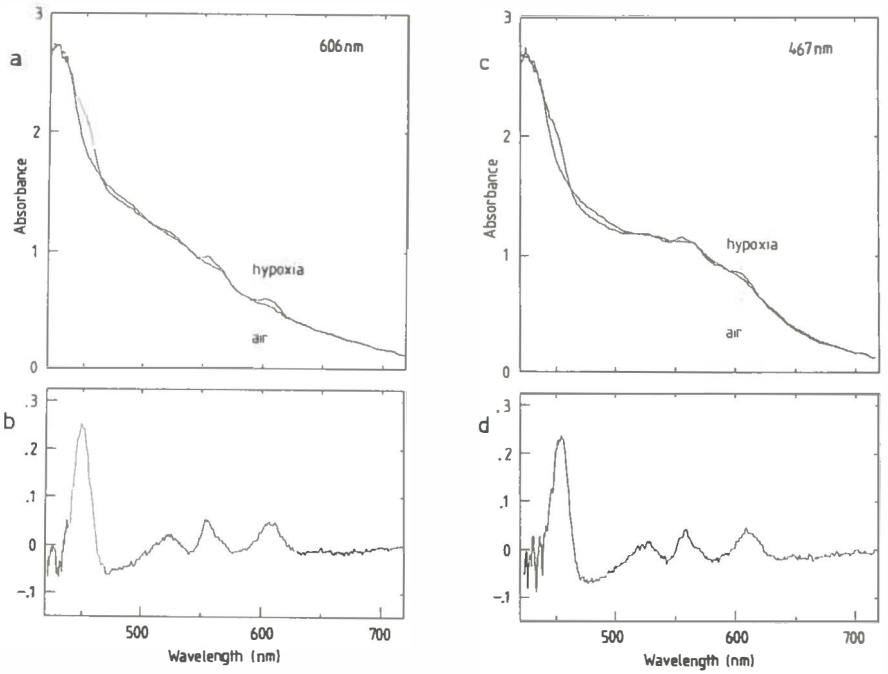

Fig. 3. Absorbance spectra of the photoreceptor layer of the blowfly eye and their absorbance difference spectra. (a) Absorbance spectra of the red (606 nm) adapted eye under normal (air) and hypoxia conditions. (b) Absorbance difference between the spectra of *a*. (c) Absorbance spectra of the blue (467 nm) adapted eye under normal (air) and hypoxia conditions. (d) Absorbance difference between the spectra of *c*.

200 facets in the dorsal part of the eye, i.e. light which has been transmitted (scattered) through the photoreceptor layer.

In the fluorescence measurements the excitation light was delivered by the objective, and the induced emission was similarly collected (epi-fluorescence).

The presented spectra (Figs 2 and 3) were from one and the same fly. For each spectrum 10 transmission runs were averaged. Each run took 15 sec. 2 min were taken in order to achieve a stable hypoxia state.

The MSP is a Leitz Orthoplan equipped with a Ploemopak illuminator and a modified Leitz Compact photometer head. The modification is a motorized interference wedge (Schott Veril S200, 400–700 nm), in front of the Hamamatsu R928 photomultiplier, thus enabling a spectral analysis of the emission beam. The objective was a Leitz NPL 10,0.20.

The excitation light was supplied by a 75W Xe-arc filtered spectrally by interference filters.

In the experiments with UV-excitation broad band illumination (Balzers K36) was applied, and the emission was measured above 400 nm (Leitz cube A). At blue-excitation narrow band light of 477 nm (Schott DAL) was applied, and emission was measured above 510 nm (Leitz cube H2). The illumination procedures as well as the spectral scans were controlled by a microcomputer (Data General MP200), which also sampled the photomultiplier signals. Processing of the spectra was performed as follows. Absorbance spectra were calculated from the transmission measurements as usual.

Emission spectra were calculated by relating the measured emissions to that of a known source, i.e. a halogen lamp (12 V, 100 W; Osram) run at 7.0 A, which is equivalent to Planck's black body at 2700 K. The spectra are given as number of quanta emitted per unit wavelength and are normalized to the peak value.

It should be known that the main visual pigment of blowflies is a xanthopsin (X, a visual pigment having 3-hydroxy retinal as chromophore; see Vogt, 1983; Vogt and Kirschfeld, 1984), which absorbs maximally in the blue-

green. Upon light absorption this state converts into a thermostable metaxanthopsin (M) state which absorbs maximally in the orange. M can be photoreconverted into X. Prolonged red light establishes a photosteady state with ≈100% xanthopsin, whereas under blue illumination the photoresteady state can lead to ≈20% X and 80% M (see e.g. Hamdorf, 1979).

RESULTS

The transmission of the photoreceptor layer of the white eyed blowfly mutant *chalky* was measured in the normal situation, i.e. in air in two photosteady states, established by 606 and 467 nm, respectively, yielding the absorbance spectra of Fig. 2a.

The absorbance difference spectrum (Fig. 2b) is the clear characteristic of the main blowfly visual pigment (see e.g. Stavenga and Schwemer, 1984).

Transmission measurements during application of nitrogen yielded the absorbance spectra presented in Fig. 2c. Since the resulting difference spectrum (Fig. 2d) is virtually identical to that measured in air (Fig. 2b) it appears that the visual pigment is not affected by hypoxia.

Figures 3a and 3c present the same spectra as Figs 2a and 2c, but they are now combined differently, namely Figs 3a and 3c represent the cases for air and hypoxia, respectively, with the visual pigment in each case being in one and the same photosteady state. Figure 3a is the case for the photosteady state established by 606 nm (high xanthopsin) and Fig. 3c that at 467 nm (low xanthopsin). The corresponding difference spectra (Fig. 3b and 3d) are virtually identical and are quite reminiscent to the redox difference spectrum of the respiratory pigments in the mitochondrial chain (Chance and Williams, 1956).

Measurement of the fluorescence from the white eyed mutant blowfly yielded the spectra of Fig. 4. Clearly upon hypoxia the UV-induced emission increases (Fig. 4a). On the other hand, blue-induced emission decreases upon hypoxia. We note that the intense excitation light established photosteady states with low xanthopsin contents well before the spectral recordings were started.

DISCUSSION

The spectral properties of fly visual pigment measured *in vivo*, with the fly in air and under

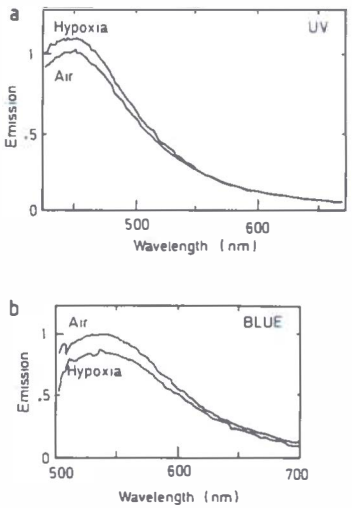

Fig. 4. Emission spectra of the blowfly compound eye, measured in air and under hypoxia, respectively. (a) UV-induced emission spectra. (b) Blue-induced emission spectra.

hypoxia, respectively, are identical in both cases (Fig. 2) and are in close agreement with previous measurements with different techniques (see e.g. Stavenga, 1976; Schwemer, 1983).

On the other hand, we found substantial absorption changes by retinal pigments upon hypoxia, which we attributed to the respiratory pigments of the mitochondria, which exist abundantly within the photoreceptor cells (e.g. Boschek, 1971; Trujillo-Cenóz, 1972). The difference spectra of Fig. 3 are very similar to those of mitochondria (e.g. Chance and Williams, 1956). The peaks appearing at 605, 555 and 445 nm are ascribed to cytochromes a, a_3 and c, c_1 and a, a_3, respectively, whereas the trough at 480 nm is attributed to flavoproteins (see also e.g. Piantidosi and Jobsis-Vandervliet, 1984). The obtained spectra varied only very slightly with eye location. Different flies also yielded insignificant variations.

We did not extend our absorption measurements into the UV. Presumably we then would have encountered a substantial absorbance increase upon hypoxia due to the reduction of NAD^+ to NADH as occurs with isolated mitochondria (Chance and Williams, 1956). This expectation seems reasonable as we found a clear sign of such a reduction in our fluorescence measurements, which show an increase in UV-induced emission (Fig. 4a). The latter phenomenon, as well as the decrease in

blue-induced green emission (Fig. 4b), which represents the reduction of flavoproteins upon hypoxia, were both investigated in detail by Chance and co-workers in isolated mitochondria (Chance and Schoener, 1966; Scholz *et al.*, 1969). Our *in vivo* spectra accord well with these studies.

We note here that substantial concentrations of cytochrome have been discovered in the ellipsosomes of fish photoreceptors (MacNichol *et al.*, 1978; Avery and Bowmaker, 1982). Also, spectra of presumably respiratory hemoproteins, measured from primate foveas, were reported by Snodderly *et al.* (1984).

The present work reinforces our hypothesis (Stavenga and Tinbergen, 1983) that redox changes of the respiratory pigments in fly photoreceptors can be measured in completely intact, living animals. The reported rapid fluorescence changes occurring upon bright light flashes (Stavenga and Tinbergen, 1983) were interpreted as resulting from transient changes in mitochondrial respiratory activity. Having characterized the spectral properties of the pigments involved we have embarked upon a further analysis of mitochondrial activity in fly photoreceptors.

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REFERENCES

Avery J. A. and Bowmaker J. K. (1982) Visual pigments in the four-eyed fish, *Anableps anableps*. *Nature* **298**, 62–63.

Boschek B. C. (1971) On the fine structure of the peripheral retina and lamina ganglionaris of the fly, *Musca domestica*. *Z. Zellforsch.* **118**, 369–409.

Chance B. and Schoener B. (1966) Fluorometric studies of flavin component of the respiratory chain. In *Flavins and Flavoproteins* (Edited by Slater E. C.), pp. 510–528. Elsevier, Amsterdam.

Chance B. and Williams G. R. (1956) The respiratory chain and oxidative phosphorylation. *Adv. Enzymol.* **17**, 65–134.

Hamdorf K. (1979) The physiology of invertebrate visual pigments. In *Handbook of Sensory Physiology* (Edited by Autrum H.), Vol. VII/6A, pp. 145–224. Springer, Berlin.

Liebman P. R. (1969) Microspectrophotometry of retinal cells. *Ann. N.Y. Acad. Sci.* **157**, 250–264.

Liebman P. A. (1972) Microspectrophotometry of photoreceptors. In *Handbook of Sensory Physiology* (Edited by Darnall H. J. A.), Vol. VII/1, pp. 481–528. Springer, Berlin.

MacNichol E. F. Jr, Kunz Y. W., Levine J. S., Harosi F. I. and Collins B. A. (1978) Ellipsosomes: Organelles containing a cytochrome-like pigment in the retinal rods of certain fishes. *Science* **200**, 549–522.

Muntz W. R. A. (1972) Inert absorbing and reflecting pigments. In *Handbook of Sensory Physiology* (Edited by Dartnall H. J. A.), Vol. VII/1, pp. 529–565. Springer, Berlin.

Piantidosi C. A. and Jobsis-Vandervliet F. F. (1984) Spectrophotometry of cerebral cytochrome *a*, a_3 in bloodless rats. *Brain Res.* **305**, 89–94.

Scholz R., Thurman R. G., Williamson J. R., Chance B. and Bucher T. (1969) Flavin and pyridine nucleotide oxidation-reduction changes in perfused rat liver. I. Anoxia and subcellular localization of fluorescent flavoproteins. *J. biol. Chem.* **9**, 2317–2324.

Schwemer J. (1983) Pathways of visual pigment regeneration in fly photoreceptor cells. *Biophys. struct. Mech.* **9**, 287–298.

Snodderly D. M., Brown P. K., Delori F. C. and Auran J. D. (1984) The macular pigment. I. Absorbance spectra, localization, and discrimination from other yellow pigments in primate retinas. *Invest. Ophthalmol. Visual Sci.* **25**, 660–673.

Stavenga D. G. (1976) Fly visual pigments. Difference in visual pigments of blowfly and dronefly peripheral retinula cells. *J. comp. Physiol.* **111**, 137–152.

Stavenga D. G. and Schwemer J. (1984) Visual pigments of invertebrates. In *Photoreception and Vision in Invertebrates* (Edited by Ali M. R. A.), pp. 11–61. Plenum, New York.

Stavenga D. G. and Tinbergen J. (1983) Light dependence of oxidative metabolism in fly compound eyes studied *in vivo* by microspectrofluorometry. *Naturwissenschaften* **70**, 618–620.

Trujillo-Cenóz O. (1972) The structural organization of the compound eye in insects. In *Handbook of Sensory Physiology* (Edited by Fuortes M. G. F.), Vol. VII/2, pp. 5–61. Springer, Berlin.

Vogt K. (1983) Is the fly visual pigment a rhodopsin? *Z. Naturforsch.* **38c**, 329–333.

Vogt K. and Kirschfeld K. (1984) Chemical identity of the chromophores of fly visual pigment. *Naturwissenschaften* **71**, 211–213.

Chapter IV

Spectral sensitivity of light induced respiratory activity of photoreceptor mitochondria in the intact fly

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Summary. Fly *Calliphora erythrocephala* (white eyed) photoreceptors were investigated in intact, living animals by microspectrofluorometry in vivo. The fluorescence of mitochondrial flavoproteins (Tinbergen and Stavenga 1986) was used to monitor transient changes in oxidative metabolism, which were induced by a test light following a stimulus of variable intensity.

Two stimulus types were applied, a brief, activating illumination and a prolonged, adapting illumination, respectively. The intensity ranges of activation and adaptation appear to be separated by ca. 3 log units.

Action spectra for inducing a criterion activation or adaptation of the light-dependent mitochondrial system are virtually indistinguishable and closely resemble the spectral sensitivity measured electrophysiologically, thus reinforcing the hypothesis (Hamdorf and Langer 1966; Stavenga and Tinbergen 1983) that the light-induced changes in oxidative metabolism in fly photoreceptors are closely linked to the phototransduction process.

On the basis of the literature we conclude that a light-induced rise in cytosolic calcium concentration is the likely cause for enhancing mitochondrial activity.

Introduction

The consumption of oxygen by blowfly retinal cells increases with illumination as was demonstrated by microrespirometry on isolated retinae (Autrum and Tscharntke 1962; Hamdorf and Kaschef 1964; Hamdorf and Schwemer 1975). Hamdorf and Langer (1966) showed that the wavelength dependence of this light-induced increase of oxygen consumption closely corresponds with the spectral sensitivity of retinal cells measured electrophysiologically.

Studying the eyes of living, intact blowflies (mutant Chalky) by microspectrofluorometry we observed transient increases in the blue-induced green emission upon illumination of dark-adapted eyes; these transients were abolished by hypoxia (Stavenga and Tinbergen 1983). We subsequently measured absorption and emission spectra from blowfly eyes in air and under hypoxia, respectively. The absorbance difference spectra clearly revealed the characteristic redox changes of the mitochondrial pigments (Tinbergen and Stavenga 1986). Furthermore, under hypoxia the blue-induced green emission was distinctly lower than in air, a typical property of mitochondrial flavoproteins as they fluoresce in the reduced state less than in the oxidized state (Chance et al. 1979).

Because, firstly, oxidation of the mitochondrial flavoproteins parallels an increased mitochondrial respiratory activity (Chance and Williams 1956) and, secondly, the time constant of the experimentally observed emission transients is similar to that of the light-induced transient increase in oxygen consumption measured in isolated retinae of blowfly (Hamdorf and Schwemer 1975) and honeybee drone (Tsacopoulos et al. 1983), we concluded that the transient increases in blue-light induced green emission reflect the transiently enhanced oxidation of mitochondrial flavoproteins in fly photoreceptor cells (see Stavenga and Tinbergen 1983; Tinbergen and Stavenga 1986). Note that anatomical studies have shown that mitochondria are numerous in photoreceptor cells (Trujillo-Cenóz 1972), thus indicating the high energy demand.

In the present paper we report action spectra of the light-dependent mitochondrial activity as observable by fluorescence. Because the action

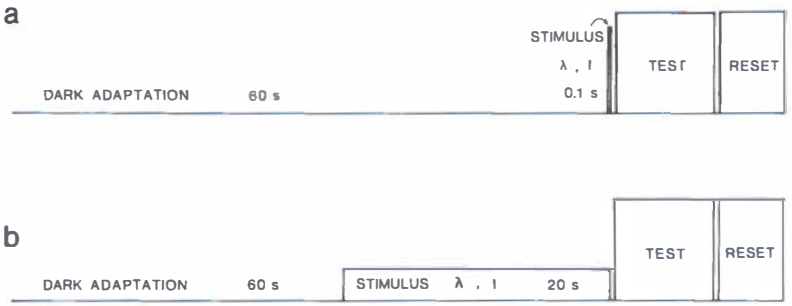

Fig. 1a, b. Diagrams of experimental procedures. **a** Activation. A dark adaptation time of 60 s preceded the stimulus, lasting 0.1 s. Subsequently a dark interval of 0.6 s was followed by a blue test light of 7.5 s. Whereas the intensity I_s and wavelength λ_s of the stimulus was variable, the test light wavelength λ_t was 477 nm and the test intensity was a constant $5.5 \cdot 10^{17}$ quanta $cm^{-2} \cdot s^{-1}$. The green emission ($\lambda_e = 550$ nm) induced by the blue test was measured. The reset illumination (590 nm, 5 s) served to reestablish the visual pigment population in the blowfly compound eye at a high level of native xanthopsin. **b** Adaptation. Duration of stimulus 20 s, but otherwise the procedure was identical to that of **a**

spectra correspond with the spectral sensitivities measured by electrophysiology and microrespirometry we conclude that microspectrofluorometry provides an attractive non-invasive method to study the role of mitochondrial respiration in photoreceptor function.

Materials and methods

Apparatus. The experiments were performed with a microspectrophotometer which consisted of a Leitz Orthoplan microscope with a Ploemopak illuminator and a Compact photometer head. The microscope objective was a Leitz NPL 10, 0.20. Epi-illumination was administered by three beams emerging from Xenon arc lamps and equipped with heat, neutral density and interference filters. The settings of intensity, wavelength and the illumination times were computer controlled (Data General MP200).

Animals. The investigated blowflies *Calliphora erythrocephala*, white eyed, mutant Chalky, were raised in the laboratory under conditions which yielded photoreceptors with a normal (high) content of visual pigment (Schwemer 1984). The animals were immobilized with wax and subsequently mounted on the Universal stage of the microspectrophotometer. A Peltier element attached to the stage controlled the temperature of the fly to a constant 15 °C.

Fluorometry. The microscope was focused at the level of the deep pseudopupil frontally in one of the eyes. The field diaphragm restricted an area of about 500 ommatidia. The green emission induced by blue (477 nm) excitation light passed a green (Balzers K 55) barrier filter and was subsequently measured by the photomultiplier (Hamamatsu R 928).

Light calibrations. Light measurements were performed with a calibrated photodiode (HUV 1000B, EG&G) at the level of the objective focus, and the intensities were subsequently calculated in quanta $cm^{-2} \cdot s^{-1}$. We note that the objective delivers the illumination intensity I_{obj} into a cone with aperture (full top angle) $\Delta\Theta = 22.6°$: when a photoreceptor with a Gaussian angular sensitivity profile, halfwidth $\Delta\rho$, is completely covered by the objective aperture then an axial point source, aligned with the photoreceptor, is equally effective when its intensity is $I_{ax} = I_{obj} (1.2\ \Delta\rho/\Delta\Theta)^2$.

Test procedures

Mitochondrial activity was tested along two different procedures, applying activating and adapting stimuli, respectively. In both procedures initially the visual pigment molecules were virtually all in the native xanthopsin state (Vogt 1983). We recall here that the main visual pigment of the blowfly in its native state maximally absorbs in the blue-green, at 490 nm (Schwemer 1984). Blue light causes photoconversion of the molecules into a thermostable, orange (580 nm) absorbing metaxanthopsin state. Prolonged red illumination results in a photosteady state with a virtually 100% xanthopsin population.

In the experiments intense blue test lights had to be applied to obtain a measurable mitochondrial fluorescence signal. Concomittantly, however, these lights induced substantial photoconversion of the visual pigment. Therefore, subsequent to the test a red reset illumination was given.

In the activation procedure (Fig. 1a) in each run first a 60 s dark period was allowed. The dark adaptation was followed by the activating light stimulus of 0.1 s duration and variable wavelength λ and intensity I. Then, after a dark interval of 0.6 s, the bright blue (477 nm) test light, lasting 7.5 s, was applied and the induced green fluorescence was registered. Finally a 5 s red (590 nm) reset was given. The next run started again with the 60 s dark period.

The adaptation procedure (Fig. 1b) only differed from the activation procedure in the duration of the stimulus which was now 20 s, a time sufficiently long to establish a state fully adapted to the stimulus.

Calculation of emission changes

Activation. The initial value of the emission signal was determined. The lowest initial value was obtained when the stimulus intensity I was $\leq 10^{13}$ quanta $cm^{-2} \cdot s^{-1}$ and increased monotonically with I (Figs. 2a, 3). The emission change with respect

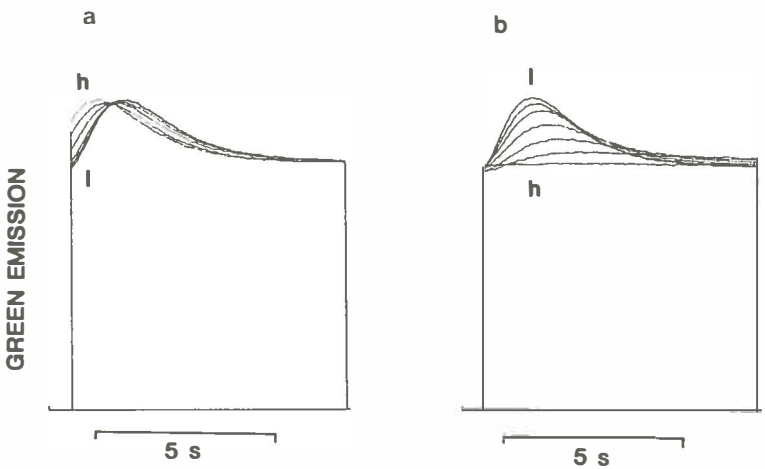

Fig. 2a, b. Recordings of the blue-induced green emission from the blowfly eye. The time course of the emission is biphasic and depends on the intensity of the preceding stimulus, which was brief (0.1 s, activation) in **a** and prolonged (20 s, adaptation) in **b**. Stimulus intensity in **a** increased from the low value $9.1 \cdot 10^{12}$ quanta $cm^{-2} \cdot s^{-1}$ (trace l) and increased via $5.1 \cdot 10^{13}$, $1.7 \cdot 10^{14}$, $5.6 \cdot 10^{14}$, $1.8 \cdot 10^{15}$ to the highest stimulus intensity $7.1 \cdot 10^{15}$ quanta $cm^{-2} \cdot s^{-1}$ (trace h). Stimulus intensity in **b** increased from $2.0 \cdot 10^{10}$ (trace l) via $6.6 \cdot 10^{10}$, $2.2 \cdot 10^{11}$, $7.2 \cdot 10^{11}$, $2.4 \cdot 10^{12}$, $2.6 \cdot 10^{13}$ to the highest value $2.8 \cdot 10^{14}$ quanta $cm^{-2} \cdot s^{-1}$ (trace h). In the activation series (**a**) the initial emission value increases with the intensity of the preceding stimulus, showing a progressively enhanced mitochondrial activity. In the adaptation series (**b**) the amplitude of the response to the test light gradually drops, showing the stronger adaptation to higher stimulus intensities

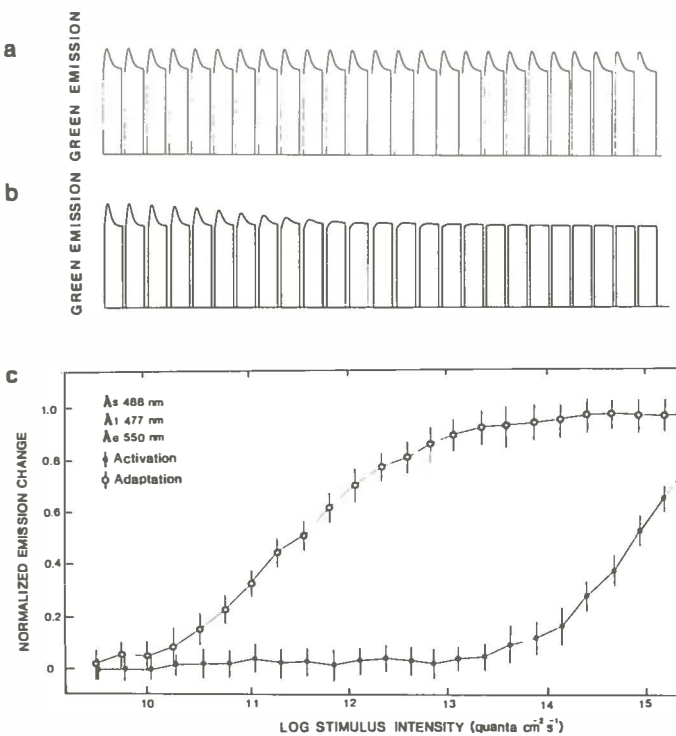

Fig. 3a–c. Intensity dependence of mitochondrial activity.
a Activation.
b Adaptation.
The blue-induced green emission curves following a series of 488 nm stimulus lights of increasing intensity are shown (compare Fig. 2a, b). From these recordings the changes in initial emission and in emission amplitude, respectively, were calculated and the values are presented normalized in **c**. The activation and adaptation series presented were of one and the same animal

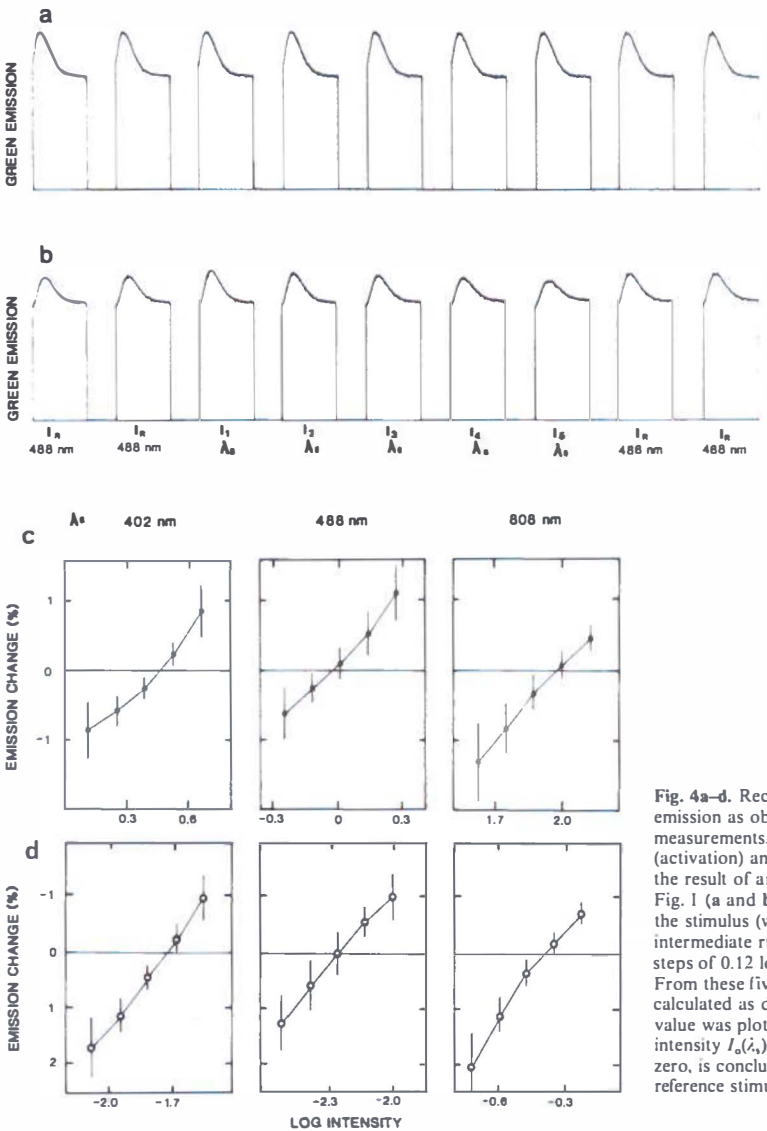

Fig. 4a–d. Recordings of blue-induced green emission as obtained in the action spectra measurements. Each of the nine recordings in **a** (activation) and **b** (adaptation), respectively, is the result of an experimental run as outlined in Fig. 1 (**a** and **b**, respectively). The intensity of the stimulus (wavelength λ_s) in the five intermediate runs 3–7 increased from I_1 to I_5 in steps of 0.12 log units. In **a** and **b** $\lambda_s = 537$ nm. From these five runs the emission change was calculated as described in the methods and its value was plotted in **c** and **d**, respectively. The intensity $I_o(\lambda_s)$, where the emission change is zero, is concluded to be equally effective as the reference stimulus

to threshold is presented normalized in Fig. 3c (closed symbols).

Adaptation. The first 3 s of the emission signal induced by the blue test light was integrated. The obtained value, $E(I)$, was maximal, E_{max}, when the adapting stimulus intensity I was $\leq 10^{10}$ quanta $cm^{-2} \cdot s^{-1}$ and decreased monitonically with increasing I to E_{min} (Figs. 2b, 3). The normalized responsiveness was calculated from $S(I) = \{E(I) - E_{min}\}/\{E_{max} - E_{min}\}$; the loss in responsiveness $\{1 - S(I)\}$ is presented in Fig. 3c as the normalized emission change (open symbols).

Estimation of action spectra

The procedures for estimating the action spectra for activation and adaptation of the light-dependent mitochondrial system were in principle identical (cf. Fig. 4a, b). At each stimulus wavelength λ_s the intensity $I_o(\lambda_s)$ was determined which was equally effective (in activating or adapting, respectively) as the chosen reference intensity I_R (of wavelength $\lambda_R = 488$ nm) being $3 \cdot 10^{14}$ quanta $cm^{-2} \cdot s^{-1}$ and $1.7 \cdot 10^{12}$ quanta $cm^{-2} \cdot s^{-1}$. Subsequently 9 runs as described under Test procedures were executed. Of these 9 runs the first and the last couple were per-

formed with the 488 nm reference stimulus. Runs 3–7 were with 5 stimulus intensities I_{1-5} differing 0.12 log units from each other; these 5 stimuli were all of the same wavelength λ_s. The intensity I_3 was chosen so that its effectivity closely approximated that of the reference stimulus ($I_3 = I_R$ when $\lambda_s = 488$ nm). With the chosen procedures it appeared possible to effectively circumvent the effects of inevitable long term drift.

From each of the 9 runs a value for the emission signal E(I) was obtained; in the case of activation the initial emission value was determined and in the case of adaptation the first 3 s of the blue-induced green emission was integrated. The emission values were further processed as follows. The 4 values obtained with the reference stimulus (i.e. the first and last couple) were averaged, yielding $\bar{E}_R$. The 5 values obtained with I_{1-5} were related to this average by calculating the relative emission change: $[E(I) - \bar{E}_R]/\bar{E}_R$.

Note that with increasing intensity the relative emission change increases in the case of activation (Fig. 4c) and decreases in the case of adaptation (Fig. 4d).

The resulting graphs for each stimulus wavelength yielded the intensity $I_o(\lambda_s)$ having equal effectivity as I_R. The action spectrum then followed from I_R/I_o.

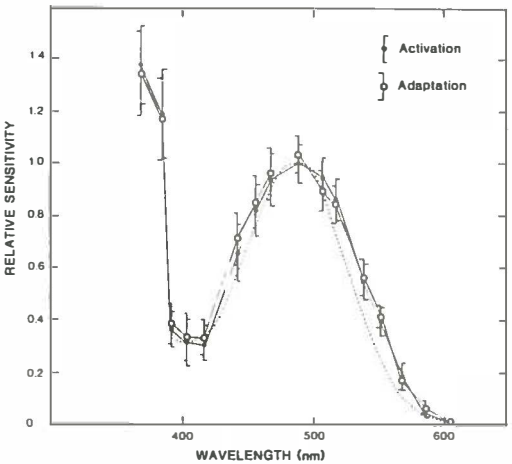

Fig. 5. Action spectra for mitochondrial activation and adaptation derived from experiments as presented in Fig. 4. Data averaged from 5 female flies. For comparison the normalized absorption spectrum of blowfly visual pigment (Schwemer 1979) is included (dotted curve). Both activation and adaptation are closely coupled to visual pigment absorption

Results

Upon illumination with an intense blue test light the dark-adapted eye of a white-eyed blowfly mutant Chalky responds with a transient increase in green emission (Fig. 2a). Rapidly (≤ 100 ms) after light-on the emission increases, peaks at 1.5 s, and gradually levels off to a plateau (cf. Stavenga and Tinbergen 1983).

The test light was preceded by 60 s darkness, the 0.1 s stimulus illumination and a 0.6 s dark interval (activation; Fig. 1a). With increasing stimulus intensity the initial value of the green emission increases (Fig. 2a), representing an increase in oxidized flavoprotein, or an increase in mitochondrial activity induced by the preceding stimulus.

The intensity dependence of this mitochondrial activation was investigated over an intensity range of well over 6 log units (Fig. 3). The stimulus intensity increased in steps of (approximately) 0.25 log units; the stimulus wavelength in Fig. 3 was 488 nm; i.e., near the peak wavelength of the sensitivity spectrum of photoreceptors R1–6 (e.g. Hardie 1985). The resulting initial emission is presented normalized as a function of the preceding stimulus intensity in Fig. 3c (closed symbols).

A prolonged illumination depresses the light sensitivity of mitochondrial activity as is shown in Fig. 3b. Here the test light was preceded by 60 s darkness, 20 s stimulus illumination and again a 0.6 s dark interval (adaptation; Fig. 1b). Clearly, the amplitude of the response to the test light diminishes progressively with increasing intensity of the preceding stimulus. In other words, the responsiveness of the system underlying mitochondrial activation decreases due to the light adapting action of the stimulus. The series of responses to the blue test light following the stimuli with intensity increasing in steps of 0.25 log units ($\lambda_s = 488$ nm) is shown in Fig. 3b. The intensity dependence of the loss in responsiveness, calculated from this series, is presented in Fig. 3c (open symbols).

Action spectra

A logical step in the analysis of a photosensitive system is the estimation of the action spectrum through a constant criterion response method (Rodieck 1973).

The exemplary cases of Fig. 4a, b show the responses to the test light of 9 consecutive runs. In runs 1, 2, 8 and 9 the stimulus wavelength was $\lambda_R = 488$ nm and the intensities were identical. In runs 3–7 the stimulus wavelength was $\lambda_s = 537$ nm and the intensity increased in steps of 0.12 log units yielding gradually changing responses for these five runs. In all experimental series 9 runs were performed. Runs 3–7 were executed with stimulus light of wavelength λ_s (including 488 nm). Runs 1, 2, 8 and 9 (with wavelength $\lambda_R = 488$ nm) served as the reference. The intensity of run 5 was made about equally effective to that of the reference stimulus. The resulting relative emission changes (see Methods) then yielded Fig. 4c, d. The action spectra (Fig. 5) were subsequently calculated as described in the Methods.

Discussion

Fluorescence from the blowfly eye

Upon illumination of a dark adapted eye bright blue light induces a transient increase in green fluorescence. We interpret this short-lived rise in emission to represent an increase in oxidized flavoproteins of the mitochondrial respiratory chain. Of course, the emission signals shown must not be taken as a quantitative measure of flavoprotein concentration. Substantial contributions to the green emission originate from at least the corneal facet lenses; also, sources of fluorescence other than the mitochondria are present in the eye tissue. We estimate that at least 30% of the steady-state emission signal is from non-mitochondrial origin.

Action spectra

Light absorption by the visual pigment molecules of blowfly photoreceptors is unequivocally involved in light-induced metabolic activity. Utilizing a microrespirometer Hamdorf and Langer (1966) measured the light-dependence of oxygen consumption by isolated blowfly retinae. Furthermore, they recorded the light-evoked generator potential. Because the action spectra of oxygen consumption and electrical signal approximated the absorption spectrum of a visual pigment (with peak wavelength $\lambda_{max}=495$ nm) they hypothesized an intimate relationship between visual pigment, phototransduction and oxygen consumption.

We have investigated the light-dependence of metabolic activity by fly photoreceptors in the eyes of completely intact, living animals with a rather indirect method, namely via the fluorescence of mitochondrial flavoproteins (Tinbergen and Stavenga 1986). We note that the rapid increase in fluorescence of oxidized flavoprotein induced by bright illumination (Figs. 2–4) conforms with the rapid increase in oxygen consumption by blowfly retinae measured microrespirometrically by Hamdorf and Schwemer (1975). The action spectrum which we find for mitochondrial activation is similar to that measured in other physiological studies on photoreceptors R 1–6 of the blowfly, as there are the spectral sensitivities of the receptor potential (Hardie 1979) and the pupillary pigment migration system (Bernard and Stavenga 1979). In these latter studies, the main peak in the blue-green originates from light absorption by blowfly xanthopsin, whereas the high UV peak is largely due to a sensitizing pigment (Kirschfeld et al. 1983; Hardie 1985). We assume that in our measurements contributions to the fluorescence signals by the central photoreceptors R 7, 8 are minor because of their small relative size and number, and thus we conclude that the action spectra of Fig. 5 are representative for the mitochondrial system of photoreceptors R 1–6.

Recently Jones and Tsacopoulos (1987) studied light-induced oxygen consumption in slices of the honeybee drone retinae using oxygen sensitive electrodes. They found that the action spectrum closely matched the absorption spectrum of the visual pigment, thus further strengthening the view that mitochondrial activity in insect photoreceptors depends on visual pigment conversion.

The finding that the action spectra for both activation and adaptation are identical bears analogy to the outcomes of electrophysiological studies by Strong and Lisman (1978; barnacle) and Lisman and Strong (1979; *Limulus*). These authors specifically investigated the question whether different pigments controlled excitation and adaptation of the receptor potential. No distinction between excitation and adaptation spectra could be observed, however (cf. Hillman et al. 1983).

A comparison of the action spectra of Fig. 5 with the spectral sensitivity curves determined by intracellular recordings from blowfly photoreceptors (e.g. Hardie 1985; Smakman and Stavenga 1986) reveals that the action spectra are less broad. Since we studied the white-eyed mutant Chalky and applied wide-field illumination a substantial fraction of light that activates (or adapts) the receptor then enters from off-axial directions. Whereas with on-axis illumination self-screening causes broadened spectral sensitivity curves off-axis light results in narrow spectra (Hardie 1985). Therefore we conclude that our action spectra are largely narrowed by the effect of off-axis (or stray) light. The narrowing effect can be easily simulated numerically and thus we estimate that the contribution by off-axis light relative to that by on-axis light was 3–5 fold, i.e. 0.5–0.7 log units.

Relations with electrophysiology and intensity dependences

Laughlin and Hardie (1978) determined from intracellular recordings of the photoreceptor potential that blowfly photoreceptors have a half-responsiveness for an axial point source $I_{ax}=4.2\cdot 10^{12}$ quanta $cm^{-2}\cdot s^{-1}$. As these photoreceptors had an acceptance angle $\Delta\rho=1.44°$ this corresponds to an intensity in our experimental arrangement (see Methods) of $I_{obj}=7.2\cdot 10^{12}$ quanta $cm^{-2}\cdot s^{-1}$. Referring to Fig. 3 it is apparent that this intensity, which half-maximally depolarizes the

photoreceptor cell, is well below the threshold for mitochondrial activation. Even more, the acceptance angle of photoreceptors in Chalky is effectively much larger than the above value for wild type flies (cf. Streck 1972). Taking the 3–5 fold contribution by stray-light into account we estimate that half-responsiveness of Chalky photoreceptors should be obtained at $I_{obj} = 2 \cdot 10^{12}$ quanta $cm^{-2} \cdot s^{-1}$, a value widely separated from the intensity range of mitochondrial activation; rather, this value is in the intensity range of mitochondrial adaptation (Fig. 3). This result is in accordance with experiments of Tsacopoulos and Poitry (1982) on the honeybee drone. They found that the amplitude of the light-induced receptor potential saturates at an intensity where the amplitude of the induced oxygen consumption is still very at a rise. Indeed, in the blowfly the intensity range of light adaptation measured electrophysiologically roughly coincides with the range of excitation of the receptor potential (Laughlin and Hardie 1978). Furthermore, the time course of dark adaptation of the receptor excitation is similar to that of mitochondrial activation (see Stavenga and Tinbergen 1983). We therefore hypothesize that the effects of adaptation measurable via mitochondrial activity are closely related to those encountered electrophysiologically.

The receptor potential saturates at intensities where mitochondrial activation becomes noticeable: $I_{ax} \geq 10^{12}$ quanta $cm^{-2} \cdot s^{-1}$ (Laughlin and Hardie 1978); i.e. $I_{obj} \geq 5 \cdot 10^{13}$ quanta $cm^{-2} \cdot s^{-1}$ in our arrangement. We have estimated (Stavenga and Tinbergen 1983) that at the intensity of half activation ($\approx 10^{15}$ quanta $cm^{-2} \cdot s^{-1}$, Fig. 3) in the order of 10^6 visual pigment molecules are converted, that number being in the order of 1% of the molecules of a photoreceptor. Thus at $5 \cdot 10^{13}$ quanta $cm^{-2} \cdot s^{-1}$ about 0.05% of the molecules are converted during the 0.1 s stimulus time. This corresponds well with data of Blumenfeld et al. (1985) who assessed in the white-eyed housefly *Musca* that the peak amplitude of the light-coincident receptor potential (LRP) is reached in ≈ 20 ms after the onset of illumination with 0.01% conversion of visual pigment, i.e. the LRP saturates with 0.05% conversion in ≈ 100 ms.

After extreme intensities a prolonged depolarizing afterpotential (PDA) emerges which is correlated with a substantial photoconversion of visual pigment molecules from the xanthopsin into the metaxanthopsin state (Minke and Kirschfeld 1984) and with a prolonged GTP-ase activity (Blumenfeld et al. 1985). Net photoconversion is substantial only at blue illuminations of $\approx 10^{15}$ quanta $cm^{-2} \cdot s^{-1}$ (see Stavenga and Tinbergen 1983) and no PDA is excited at long wavelengths. Because mitochondrial activity is light-induced at all wavelengths we conclude that the PDA is not a direct cause of mitochondrial activation.

The cause of mitochondrial activation

An understanding of the cellular processes leading ultimately to mitochondrial activation can be derived from fundamental photoreceptor studies performed predominantly on the honeybee drone and the horseshoe crab *Limulus*. Firstly, visual pigment conversion results in an increased membrane permeability for ions. Sodium ions then massively rush into the photoreceptor as shown by Coles and Orkand (1982) and Tsacopoulos et al. (1983); see also Fain and Lisman (1981). Because the increased intracellular ion concentration activates the sodium pump, which requires metabolic energy, an obvious conjecture is that the increased pump activity results in an increased ADP concentration and thus enhances mitochondrial activation (see e.g. Sacktor 1975). However, oxygen consumption and therefore mitochondrial activity precedes sodium pumping and thus Tsacopoulos et al. (1983) concluded that mitochondrial respiration must be stimulated by some signal which is generated earlier than the rise in ADP produced by the Na-pump. More recently, Coles et al. (1984) measured the concentration of ATP in the retina of the honeybee drone subsequent to a bright flash and obtained the startling result that [ATP] transiently rises with a time course similar to that of the light-induced oxygen consumption. Furthermore, Tsacopoulos et al. (1986) measured on a single *Limulus* ventral photoreceptor cell a clear light-induced increase in oxygen consumption, but they found no change in oxygen consumption upon injection of sodium ions, although recordings of the membrane potential clearly demonstrated activation of the electrogenic sodium pump. The latter authors therefore concluded that neither an increase in $[Na^+]$, nor an increase in [ADP] is the main stimulus of mitochondrial respiration after a light flash and that 'A role for cytosolic Ca cannot be excluded'.

Indeed, it appears that calcium is the principal candidate for both mitochondrial activation and adaptation. Intense, brief illuminations cause a transient rise in intracellular calcium concentration (e.g. Brown and Blinks 1974; Levy and Fein 1985). This Ca_i is released from an intracellular compartment (see Brown and Blinks 1974) in the most light sensitive part of the photoreceptor cell (Levy and

Fein 1985) and presumably from the subrhabdomeric smooth endoplasmic reticulum (SER) cisternae (Lisman and Strong 1979) which actively accumulate Ca^{++} (e.g. Walz 1983). The endoplasmic reticulum generally appears to be the main organelle regulating the cytoplasmic calcium concentration in non-muscle cells (Somlyo et al. 1985).

The light-induced increase in Ca_i participates in both excitation of the receptor potential and its adaptation (Bolsover and Brown 1985; Payne et al. 1986a). The causal link between visual pigment conversion and the rise in Ca_i probably is an increase in inositol trisphosphate, Ins (1,4,5) P_3 (Brown et al. 1984; Fein et al. 1984), which is released by hydrolysis of $PtdInsP_2$ and which in turn releases calcium from a non-mitochondrial store (review Berridge and Irvine 1984). Brown and Rubin (1984) and Payne et al. (1986b) have directly demonstrated in the ventral photoreceptor of *Limulus* that $InsP_3$ causes a rise in intracellular calcium and that this rise is necessary for excitation and adaptation of the photoreceptor by Ins(1,4,5) P_3 (Fein 1986; Payne et al. 1986b).

Because the oxygen consumption by mitochondria of blowfly flight muscle is activated by Ca^{++} (see Sacktor 1975) we presume that also in blowfly photoreceptors the light-induced rise in cytosolic calcium enhances mitochondrial activity. Prolonged illumination and increased calcium concentration results in adaptation of the blowfly photoreceptor (see Muijser 1979).

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References

Autrum H, Tscharntke H (1962) Der Sauerstoffverbrauch der Insektenretina im Licht und im Dunkeln. Z Vergl Physiol 45:695–710

Bernard GD, Stavenga DG (1979) Spectral sensitivities of retinular cells measured in intact living flies by an optical method. J Comp Physiol 134:95–107

Berridge MJ, Irvine RF (1984) Inositol trisphosphate, a novel second messenger in cellular signal transduction. Nature 312:315–321

Blumenfeld A, Erusalimsky J, Heichal O, Selinger Z, Minke B (1985) Light-activated guanosinetriphosphate in *Musca* eye membranes resembles the prolonged depolarizing after potential in photoreceptor cells. Proc Natl Acad Sci USA 82:7116–7120

Bolsover SR, Brown JE (1985) Calcium ion, an intracellular messenger of light adaptation, also participates in excitation of *Limulus* photoreceptors. J Physiol 364:381–393

Brown JE, Blinks JR (1974) Changes in intracellular free calcium concentration during illumination of invertebrate photoreceptors. J Gen Physiol 64:643–665

Brown JE, Rubin LJ (1984) A direct demonstration that inositol-trisphosphate induces an increase in intracellular calcium in *Limulus* photoreceptors. Biochem Biophys Res Comm 125:1137–1142

Brown JE, Rubin LJ, Ghalayini AJ, Tarver AL, Irvine RF, Berridge MJ, Anderson RE (1984) Myo-Inositol polyphosphate may be a messenger for visual excitation in *Limulus* photoreceptors. Nature 311:160–162

Chance B, Williams GR (1956) The respiratory chain and oxidative phosphorylation. Adv Enzymol 17:65–134

Chance B, Schoener B, Oshino R, Itshak F, Nakase Y (1979) Oxidation-reduction ratio studies of mitochondria in freeze-trapped samples. J Biol Chem 254:4764–4771

Coles JA, Orkand RK (1982) Sodium activity in drone photoreceptors. J Physiol (Lond) 332:16P–17P

Coles JA, Tsacopoulos M, Dunant Y (1984) Régulation de l'extra-consommation d'O_2 par les photorécepteurs du faux bourdon à la suite d'un flash de lumière. Klin Mbl Augenheilk 184:332–333

Fain GL, Lisman JE (1981) Membrane conductances of photoreceptors. Progr Biophys Molec Biol 37:91–147

Fein A (1986) Excitation and adaptation of *Limulus* photoreceptors by light and inositol 1,4,5-trisphosphate. Trends Neurosci 9:110–114

Fein A, Payne R, Corson DW, Berridge MJ, Irvine RF (1984) Photoreceptor excitation and adaptation by inositol 1,4,5 trisphosphate. Nature 311:157–160

Hamdorf K, Kaschef AH (1964) Der Sauerstoffverbrauch des Facettenauges von *Calliphora erythrocephala* in Abhängigkeit von der Temperatur und dem Ionenmilieu. Z Vergl Physiol 48:251–265

Hamdorf K, Langer H (1966) Der Sauerstoffverbrauch des Facettenauges von *Calliphora erythrocephala* in Abhängigkeit von der Wellenlänge des Reizlichtes. Z Vergl Physiol 52:386–400

Hamdorf K, Schwemer J (1975) Photoregeneration and the adaptation process in insect photoreceptors. In: Snyder AW, Menzel R (eds) Photoreceptor optics. Springer, Berlin Heidelberg New York, pp 363–389

Hardie RC (1979) Electrophysiological analysis of fly retina. I. Comparative properties of R1–6 and R7 and 8. J Comp Physiol 129:19–33

Hardie RC (1985) Functional organization of the fly retina. Progr Sens Physiol 5:1–79

Hillman P, Hochstein S, Minke B (1983) Transduction in invertebrate photoreceptors: Role of pigment bistability. Physiol Rev 63:668–772

Jones GJ, Tsacopoulos M (1987) The response to coloured light flashes of the oxygen consumption of the honeybee drone retina. J Gen Physiol (in press)

Kirschfeld K, Feiler R, Hardie R, Vogt K, Franceschini N (1983) The sensitizing pigment in fly photoreceptors. Properties and candidates. Biophys Struct Mech 10:81–92

Laughlin SB, Hardie RC (1978) Common strategies for light adaptation in the peripheral visual systems of fly and dragonfly. J Comp Physiol 128:319–340

Levy S, Fein A (1985) Relationship between light sensitivity and intracellular free Ca concentration in *Limulus* ventral photoreceptors. J Gen Physiol 85:805–841

Lisman JE, Strong JA (1979) The initiation of excitation and light adaptation in *Limulus* ventral photoreceptors. J Gen Physiol 73:219–243

Minke B, Kirschfeld K (1984) Non-local interactions between

light induced processes in *Calliphora* photoreceptors. J Comp Physiol A 154:175–187
Muijser H (1979) The receptor potential of retinular cells of the blowfly *Calliphora:* the role of sodium, potassium and calcium ions. J Comp Physiol 132:87–95
Payne R, Corson DW, Fein A (1986a) Pressure injection of calcium both excites and adapts *Limulus* ventral photoreceptors. J Gen Physiol 88:107–126
Payne R, Corson DW, Fein A, Berridge MJ (1986b) Excitation and adaptation of *Limulus* ventral photoreceptors by inositol (1,4,5) trisphosphate result from a rise in intracellular calcium. J Gen Physiol 88:127–142
Rodieck RW (1973) The vertebrate retina. Principles of structure and function. Freeman, San Francisco
Sacktor B (1975) Biochemistry of insect flight. In: Candy DJ, Kilby BA (eds) Insect biochemistry and function. Chapman and Hall, London, pp 1–88
Schwemer J (1979) Molekulare Grundlagen der Photorezeption bei der Schmeißfliege *Calliphora erythrocephala* Meig. Habilitationsschrift, Bochum
Schwemer J (1984) Renewal of visual pigment in photoreceptors of the blowfly. J Comp Physiol A 154:535–547
Smakman JGJ, Stavenga DG (1986) Spectral sensitivity of blowfly photoreceptors: dependence on waveguide effects and pigment concentration. Vision Res 7:1019–1025
Somlyo AP, Bond M, Somlyo AV (1985) Calcium content of mitochondria and endoplasmic reticulum in liver frozen rapidly in vivo. Nature 314:622–625
Stavenga DG, Tinbergen J (1983) Light dependence of oxidative metabolism in fly compound eyes studied in vivo by microspectrofluorometry. Naturwissenschaften 70:618–620
Streck P (1972) Der Einfluß des Schirmpigmentes auf das Sehfeld einzelner Sehzellen der Fliege *Calliphora erythrocephala* Meig. Z Vergl Physiol 76:372–402
Strong JA, Lisman J (1978) Initiation of light adaptation in barnacle photoreceptors. Science 200:1485–1487
Tinbergen J, Stavenga DG (1986) Photoreceptor redox state monitored in vivo by transmission and fluorescence microspectrophotometry in blowfly compound eyes. Vision Res 26:239–243
Trujillo-Cenóz O (1972) The structural organization of the compound eye in insects. In: Fuortes MGF (ed), Handbook of sensory physiology, vol VII/2. Springer, Berlin Heidelberg New York, pp 5–61
Tsacopoulos M, Poitry S (1982) Kinetics of oxygen consumption after a single flash of light in photoreceptors of the drone (*Apis mellifera*). J Gen Physiol 80:19–55
Tsacopoulos M, Orkand RK, Coles JA, Levy S, Poitry S (1983) Oxygen uptake occurs faster than sodium pumping in bee retina after a light flash. Nature 301:604–606
Tsacopoulos M, Fein A, Poitry S (1986) Stimulus-induced increase of mitochondrial respiration in a single neuron. Experientia 42:642
Vogt K (1983) Is the fly visual pigment a rhodopsin? Z Naturforsch 38C:329–333
Walz B (1983) Calcium-sequestering smooth endoplasmic reticulum in retinula cells of the blowfly. J Ultrastruct Res 81:240–248

Chapter V

THE LANDING RESPONSE OF THE BLOWFLY

In flying insects flight motor output stabilizes or alters the position and orientation of the animal in the environment. The often complex sensory input and the subsequent rapid analysis by the neuronal system play a crucial role in the control of flight behaviour. The speed at which decisions have to be made increases with the flight velocity and the degree of freedom of the animal. Moreover, many different behavioural tasks are combined into a consistent flight behaviour in which the decision to terminate flight and execute a landing is an essential component.

When an insect is landing it reduces its flight speed, and ultimately the unfolded legs make contact with the substrate. After the apparent decision to execute a landing a rather complex and variable behavioural pattern can be observed, in which generally substrate-oriented response components play an important role. In the case of an unsuccessful attempt the animal even may return to the same spot in order to retry to land at that particular place. During flight the visual environment may suddenly change and under such an unexpected situation the animal may be forced to terminate flight. Then an attempt to perform a landing directly follows, nearly without substrate oriented response components. Probably, this same type of behavioural response is elicited in tethered, stationary flying flies, upon sufficient movement of the environment or sudden intensity changes.

The landing response of stationary flying blowflies.

Analysis of the landing response is obviously difficult to perform on free flying animals (Wagner 1982). On the contrary, the landing response of tethered animals can be relatively easily studied, especially in flies as described in the following. Blowflies, *Calliphora erythrocephala*, were prepared for stationary flight by connecting a small piece of aluminum wire to the dorsal side of both the thorax and the head. The animals were then mounted in the experimental set up. They were thus kept stationary for observation and measurements. Also the field of vision was stationary, because fly eye movements are due to head movements. The orientation of the visual field of the fly was aligned with the equipment by using a swing-in epi-illumination microscope, and observing the equatorial deep pseudopupils of both compound eyes (Franceschini and Kirschfeld 1971). After the alignment of the visual field the microscope was removed. An

Fig. 1. Stationary flight of Calliphora at observation from aside. The legs held in a characteristic way. Due to multi-flash illumination both extreme wing positions are visible.

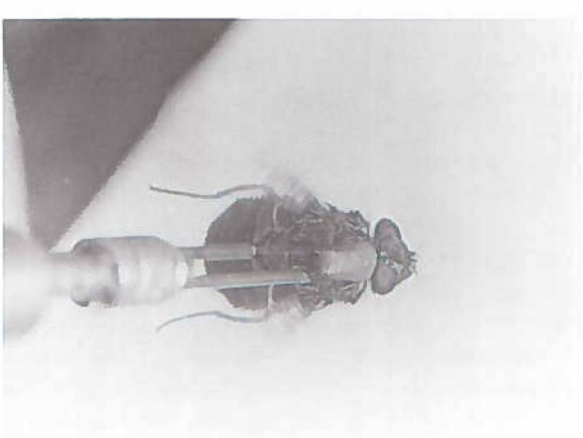


Fig. 2. Stationary flight of Calliphora at observation from above. The hind legs are clearly visible beside and behind the abdomen. In the space between head and thorax the fore leg tibia, located just in front of the thorax, are visible also.

airstream was directed to the antennae in order to improve flight stability. It thus proved that tethered flies start flight as soon as the tactile contact between the legs and the substrate is removed.

During flight the wings beat at a frequency of ≈ 140 - 170 Hz and under an angle of ≈ 30 - 50° with the body axis, i.e. in a plane from below the head to above the abdomen. Directly after take-off the legs become positioned in a characteristic way. In this special flight posture femur and tibia of the fore legs are folded at either side of the body and held in the space between head and thorax, the fore leg tarsi point forward just below the head; the middle leg femur and tibia are folded similarly in the space between thorax and abdomen, the tarsi point forward below the abdomen; the hind legs are not folded, but oriented backward with the femur slightly upward, the tibia beside and the tarsi behind the abdomen. This posture improves the streamline of the body during flight. The position of the legs is illustrated in Figs. 1 and 2.

In response to visual stimulation the fly may suddenly execute a characteristic unfolding of the legs, indicating that the animal has changed the behaviour from flight to execution of the landing response (Goodman 1960). In the landing response the fore legs move upward and forward just beside the head, predominantly due to the stretching of the joints at either side of the tibia. The middle legs move downward and finally also forward, mainly due to the stretching of the joints at either side of femur and tibia. The hind legs are lowered by a downward rotation. In the movement of all legs also a lateral outward component is present. Finally then, the unfolded legs provide the fly a stable undercarriage.

The leg positions at moments when the unfolding is approximately complete is illustrated in Figs. 3 and 4. Figs. 5 and 6, taken under multi-flash stroboscopic illumination, visualize successive leg positions during the unfolding of the legs. From these and similar pictures it has been obtained that the complete unfolding of the legs takes 40 - 50 ms in *Calliphora*, a slightly longer time span than in *Musca*, where 30 - 40 ms has been measured (Borst 1986).

Upon execution of the landing response not only leg movements occur, but also a sudden change of the wing beat (cf. Goodman 1960). Direct observations, partly under stroboscopic illumination conditions, indicate that both stroke angle and stroke amplitude increase, that the wing beat frequency decreases and that the angle of attack is suddenly altered. Furthermore, the fly attempts to change the orientation of the body, in which the ventral side and the unfolding legs are rotated in the direction of flight. The different components of the changing wing action and the change in the orientation of the body together will alter flight lift and thrust forces and cause deceleration of the animal. No indications have been noticed that leg movements have a speciai phase relation with the wing beat

Fig. 3. Side view of Calliphora with the legs unfolded at execution of the landing response. The wings are positioned frontally.

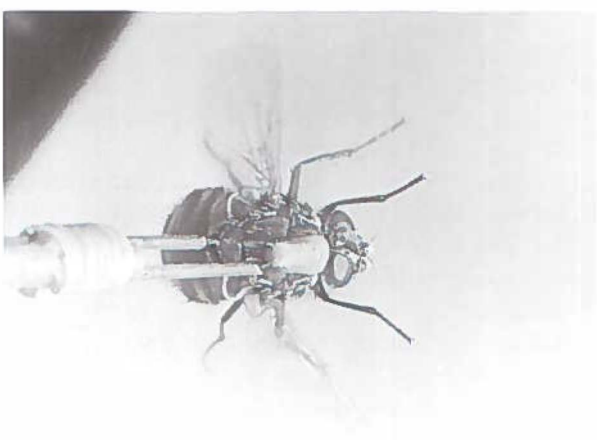


Fig. 4. Calliphora with the legs unfolded at execution of the landing response, observation from above.

Fig. 5. Different phases of the unfolding of the legs taken under multi-flash illumination conditions. Flash intervals of 3.5 ms. In this poorly flying animal the wings did not reach frontal positions, leaving the successive leg positions better visible.

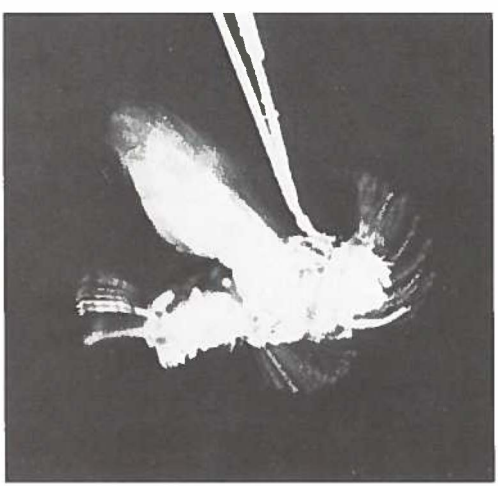


Fig. 6. The unfolding of the legs taken under multi-flash illumination conditions. Flash intervals of 3.5 ms. Both wings and legs did not move entirely symmetrically. Poorly flying animal in which the wings did not reach frontal positions.

cycle. When the landing response is induced by stimuli of short duration, the different response components start at nearly identical moments. Subthreshold stimuli for the landing response may induce changes in wing action being components of different behavioural response patterns.

Execution of the landing response mediates a rapid speed reduction with a final deceleration by the unfolded legs at contact with the substrate.

Measuring the landing response.

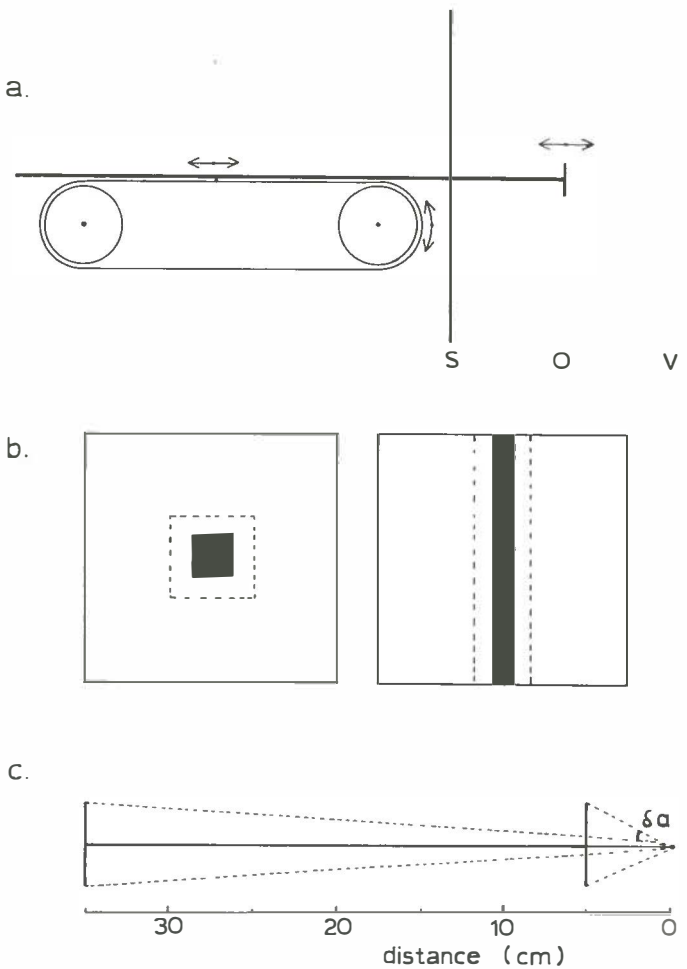
Borst and Bahde (1986,1987) have used a method of optical detection in which the movement of the fore legs was measured. In the present work a slightly different detection method was used. Exclusively movement of the right fore leg was detected by an optical device, in which a 5 cm long narrow beam of light was directed to a photodiode. The movement of the foreleg, of which a constant phase modulated the intensity of the detector beam, was registered by the diode. The beam was oriented vertically and positioned in front of the right compound eye, just besides the head, near the antenna. At this beam position and orientation the moving wing did not activate the detector, and the unfolding of the fore leg was detected at a moment, which was only a few ms delayed from the start of the fore leg movement.

The moment at which the leg movement starts is not entirely identical to the moment at which the visual stimulus is presented. The response is slightly delayed with respect to the stimulus and is detected 35 ± 5 ms after the presentation of the adequate stimulus. Responses to different types of visual stimuli all demonstrated that no response is measured later than 45 ms after the termination of the stimulus. Consequently, a landing response state in which the legs are fully unfolded is reached within 0.1 s, due to the duration of the delay (35 ± 5 ms) and of the unfolding of the legs (45 ± 5 ms).

Visual stimuli eliciting the landing response.

Visual stimuli consisting of changes in light intensity and a great variety of movement stimuli are sufficient to elicit the landing response. In a few cases the response to moving stimuli seemed to depend directly on the decrease in distance of the stimulus to the fly and therefore suggested the involvement of binocular distance estimation. Moving stimuli which do not change in their distance to the fly elicit the landing response very effectively.

Fig. 7. Approaching objects, stimulus set up. a. Object (O) in front of screen (S) moves toward or away from the stationary flying fly (V). The object is mounted at the tip of a bar, which is connected to a beld driven by a stepping motor. The displacement of the object amounts 1 mm each 0.8° step of the motor. Maximum velocity of the movement 30 cm/s and maximum distance 30 cm. Directly after the movement toward the fly the direction of the movement was reversed and the object moved back to the start position. b. Dark objects in front of bright screen. Dotted lines: after the approach the angular size increases. c. Angular positions of a 5 cm object before and after movement over 30 cm from 35 cm to 5 cm. δa indicates the change in angular position of the edges.



1 Responses to changes in light intensity.

The landing response can be elicited by a decrease in ambient light intensity. Responses are executed depending on the relative decrease in intensity, but also depending on the rate of the change (*Lucilia*, Goodman 1960). In *Lucilia* upon an increase in light intensity no response occurred. In *Musca* the landing response to light-off has shown to occur very rapidly: the leg movements start just 50 ms after stimulus presentation (Borst 1986). Similarly, the light-off stimulus is an adequate stimulus in *Calliphora*. However, *Calliphora* also responds to a sudden increase in intensity, when presented as an intense flash of short duration (0.1 ms).

2 Moving stimuli.

2.1 Responses to approaching objects.

A further type of stimulus eliciting the landing response is the approaching movement of two-dimensional simple objects. The approach of these objects simulates rather realistic and simple landing situations. Responses might directly depend on the change in distance and/or on the angular characteristics of the approaching movement, but, at least in part, also by the changes in intensity resulting from the movement of the objects.

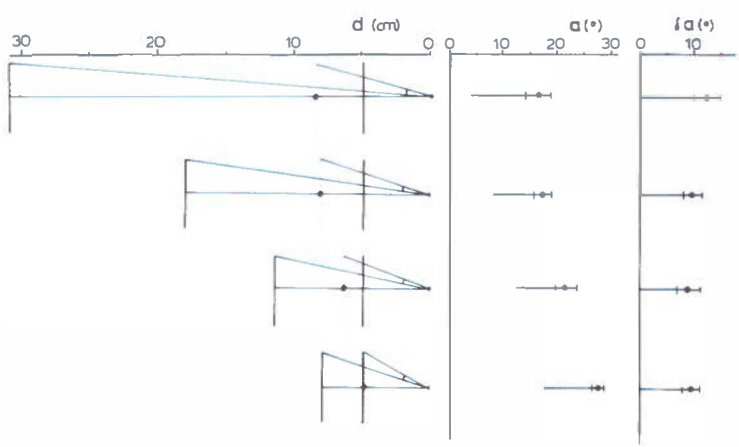


Fig. 8. Approaching object of 5 cm moving at 30 cm/s and starting from different distances. O indicates the average response distance. α and δα indicate the angular position and change in the angular position of the object edges until the average response O

Goodman (1960) studied the landing response of the blowfly *Lucilia* with dark approaching objects in front of a bright screen, moving at constant velocity from the direction of flight toward the stationary flying fly. The elicited landing responses depended on the velocity of the movement, the size of the object and on the distance travelled by the object. The movement sufficient to elicit the response was not directly determined by the distance over which the object travelled, but by the change in angular size of the object. The change in angular size eliciting a response in 100% of the tests was constant at each velocity of approach and decreased with increasing velocity, independent of the object size (120 - 20° in the range 15 - 65 cm/s). In Goodman's experiments both distance and object size were varied at the same time.

Experiments with approaching objects similar to those of Goodman (1960) were performed on *Calliphora* with the apparatus of Fig. 7. In the experimental arrangement of Fig. 8 the object moved toward the fly at constant velocity of approach and started from different distances. The elicited responses, however, appeared to depend on the distance at which the movement started: the change in angular size of the object was not constant. Evidently, the responses to the approaching movement occurred after an angular displacement of the object edges which depended on the time required for the angular displacement. The results suggest that the response is angular velocity dependent and that lower angular velocities contribute less than higher angular velocities. Low sensitivity to low angular velocity implies that at lower velocities of approach the landing response is elicited after a larger change in angular size of the object.

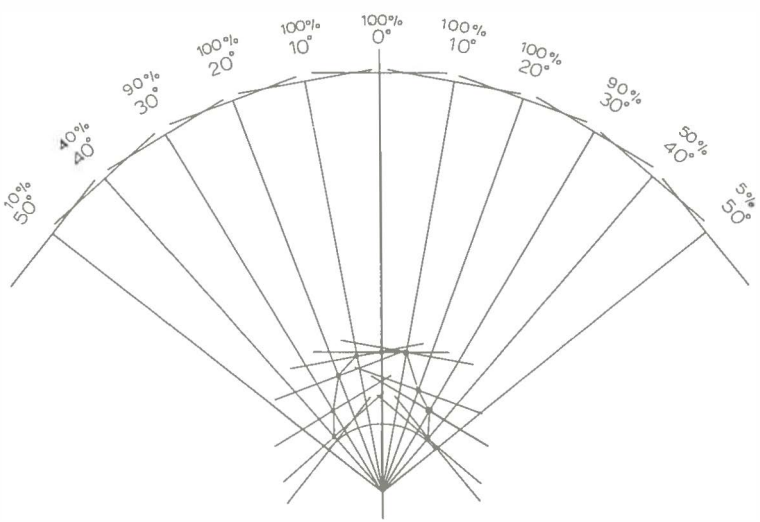


Fig. 9. Approaching movement from different directions in the horizontal plane.

Dark, bright and textured objects elicit responses at nearly identical distances, indicating that predominantly the outer edges of the object determine the response, at least when the angular size of the object does not exceed ≈45° (cf. Eckert and Hamdorf 1980).

Objects approaching from oblique directions elicited responses at different distances depending on the angle of approach. Movement toward the fly starting at more lateral positions resulted in responses which were executed later, i.e. at a shorter distance to the fly. As is illustrated in Fig. 9 the response distance was nearly identical for the movement presented from frontally and 10° laterally; the response distance was reduced at movement from 20° laterally, corresponding with a larger angular displacement of the contrast borders and indicating a lower sensitivity. Also, the response percentage was reduced when the movement toward the fly started more laterally. These results suggest that movement sensitivity is high when both eyes are stimulated. Similarly, when one eye of the fly is covered by black paint the sensitivity to approaching movement presented frontally was greatly reduced compared to the normal binocular situation. Approaching movement presented from directions above and below the equator showed differences in sensitivity also. The largest response distances and high response percentages were found for approaching movement from directions just below the equator of the compound eyes (Fig. 10).

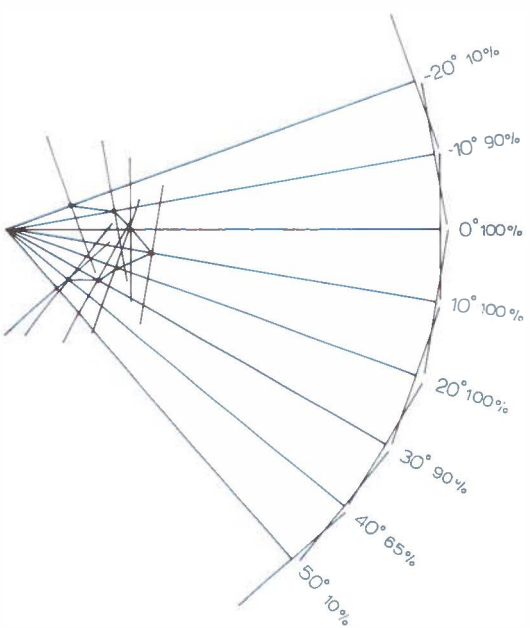


Fig. 10. Approaching movement from different directions in the vertical plane.

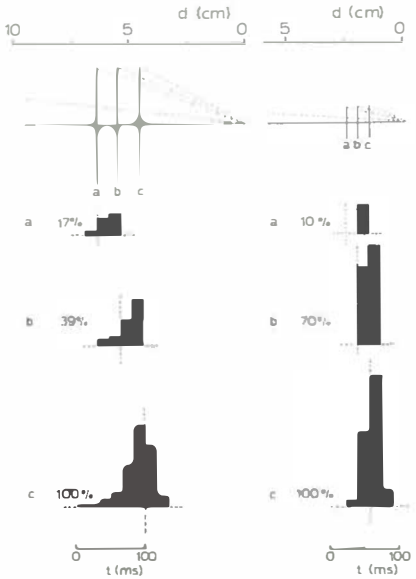


Fig. 11. Response delay. Objects approaching until positions a. b. and c. at movement of 30 cm/s . Histograms indicate response percentages and response moments.Responses were generally given just after the object had reached the closest distance to the fly.

The responses to the approaching movement are executed always just later than the stimulus has been presented, as is illustrated in Fig. 11 where the objects moved over slightly varying distances. The visual processing and motor commands take on the average approximately 35 ms.

Approaching movement of rectangular objects which are very large in the vertical direction produces expansion in exclusively horizontal directions (Fig. 7b) and show that horizontal movement is sufficient to elicit the landing response. With objects of different horizontal width, consisting of stripes, Eckert and Hamdorf (1980) have shown that the sensitivity to approaching movement is higher in the middle part of the visual field than in slightly more lateral parts: small objects of 1 cm width induced responses at a distance corresponding with an angular size of 13.2°, whereas larger objects (3 - 6 cm width) elicited responses at distances corresponding to an angular size of 29.5 °. This difference in response angle for small objects is attributed to a higher sensitivity in the binocular overlap area of the field of vision. Eckert and Hamdorf (1980) have hypothesized that possibly binocular distance estimation plays a role in eliciting the landing response at shorter distances (see also Beersma et al. 1977; Burkhardt et al. 1973).

2.2 Responses to patterns moving at a fixed distance to the fly.

The change in distance between the visual stimulus and the fly is not essential for moving stimuli to elicit the landing response (e.g. Goodman 1960; Braitenberg and Taddei 1967). Moving patterns which remain at a constant distance to the animal are often very effective stimuli. Experiments with these stimuli provide further evidence that the processing of distance information is generally not used.

2.2.1 Rotating spiral patterns.

A rather complex class of moving stimuli which do not change in distance to the fly are rotating linear spiral patterns (Fig. 12), which are effective depending on the direction of the rotation. When presented in the

Fig. 12. Linear spiral pattern consisting of 8 spiral arms of equal radial width and constant pitch. At clockwise rotation around the center, the contrast borders in the pattern exhibit an expanding, centrifugal movement, having a radial expansion velocity directly proportional to the rotation speed and the pitch of the spiral arms. The movement has aspects in common with the approach of an object. Counterclockwise rotation induces contracting, centripetal movement of the contrast borders, having aspects in common with an object moving away. The rotation of the patterns induces a tangential movement component of the contrast borders also, which decreases toward the periphery of the pattern.

Fig. 13. Exponential spiral pattern consisting of 8 spiral arms of exponentially increasing radial width and pitch. Clockwise rotation around the center induces expanding radial movement of the contrast borders in the pattern, which is directly related with the rotation speed and increases exponentially toward the periphery of the pattern, and in addition a tangential movement component. The movement has aspects in common with the approach of an object. Counterclockwise rotation induces movement in the opposite direction, having aspects in common with an object moving away.

medio-frontal part of the visual field the expanding movement of the contrast borders elicits the landing response depending on the angular characteristics and on the rotation speed of the pattern (Fernandez and Taddei 1970; Taddei and Fernandez 1973a,b; Fernandez and Taddei 1975). Responses predominantly depend on the radial angular velocity, but the tangential velocities play an additional role. Furthermore, a decrease in spatial wavelength and an increase in contrast frequency shift the threshold rotation speed to a higher value. Movement in the opposite direction, i.e. contracting movement of the spirals, does not elicit the landing response, but induce accelerations of the animal, as was concluded from changes in the wing action.

Especially the spiral patterns which are presented approximately coaxial with the direction of flight are effective stimuli in eliciting the landing response. Those presented in different parts of the visual field reveal that the directions of movement in the spiral pattern generally do not fit with the movement sensitivities of the visual system. Varying angular velocities presented simultaneously in different parts of the visual field provide sufficient movement to elicit the landing response also: rotating exponential spiral patterns (Fig. 13) can elicit the landing response. The highest angular velocities of the movement, which are generated in the periphery of the pattern, predominantly determine the threshold rotation speeds of the responses to this type of pattern. An extreme type of spiral pattern which also can induce landing responses is a radial pattern having a pitch = ∞ : i.e. tangential movement components are sufficient to elicit the landing response.

Movement in nearly exclusively one direction has been presented using partly covered rotating spirals. The responses to varying direction of movement showed lower threshold velocities at vertical movement than at horizontal movement, which in monocular flies was the opposite (Taddei and Fernandez 1973), as was confirmed by using the type of pattern presented below.

2.2.2 Moving periodic and stripe patterns.

Especially patterns moving in one specific direction, and which do not change in the distance to the fly, have been applied to investigate the movement sensitivity of the landing response and the dependence on the direction of the movement (e.g. Eckert et al. 1979; Eckert 1980,1981,1983; Eckert and Hamdorf 1980,1981,1983; Wehrhahn et al. 1981; Borst 1986, Borst and Bahde 1986,1987). The spatial configuration of these patterns consists of simple dark and bright stripes of equal width, periodic sinewave patterns and single or double contrasting stripes, which all moved in the direction perpendicular to the contrast borders.

Landing responses have been elicited depending on, predominantly, the angular velocity of the movement, the direction of the movement and the position of the movement stimulus in the visual field, and furthermore also depending on the spatial pattern wavelength or stripe width, the contrast and the size of the stimulated area. Often two movement stimuli were presented simultaneously or successively in order to investigate excitatory and inhibitory contributions to the landing response and their interactions depending on the direction of the movement and the position in the visual field. Most of the experiments have been performed with stimuli presented in the equatorial eye region, i.e. at and around the line of mirror symmetry between upper and lower halves of both compound eyes. In the equatorial parts of the visual field the front to back direction of movement can elicit the landing response and therefore is excitatory (Eckert and Hamdorf 1980; Wehrhahn et al. 1981; Taddei and Fernandez 1973). The opposite back to front inward direction of movement is not indifferent to the landing response but inhibitory, as follows from simultaneous or successive presentation of horizontal inward and horizontal outward movement (Eckert et al. 1979; Eckert 1980; Eckert and Hamdorf 1981; Wehrhahn et al. 1981). The directions of movement sensitivity are arranged mirror symmetrically for left and right eye. The preference direction is oriented along the H- (or Z-axis) of the ommatidial raster of the compound eye (Eckert and Hamdorf 1983, Eckert 1983). The main directions of sensitivity are arranged approximately according to the directions in the flow field at forward flight (Collett 1980). The pole is probably not located at the equatorial median plane, but slightly below the equator , $\approx 10°$. However, the preference direction is not the only direction of sensitivity, the perpendicular directions of movement are excitatory also, at least when the periodic patterns are used (Eckert and Hamdorf 1983; Eckert 1983). Similarly, the responsivity to horizontal outward movement presented to one eye is lower than to the combination with horizontal outward, upward and downward movement presented to the other eye (Wehrhahn et al.1981).

Periodic patterns moving in the horizontal outward direction induce in the monocular visual field responses with a contrast frequency optimum at 6 - 8 Hz (Eckert 1980; Eckert and Hamdorf 1981; Wehrhahn et al. 1981). This value is higher than the 1 - 3 Hz generally found for the optomotor torque response (e.g. Eckert and Hamdorf 1981). The measurements by Borst and Bahde (1987),obtained from the landing response latencies, indicate that the landing response at low contrast and the optomotor torque response at high contrast have nearly identical contrast frequency optima,between 1 and 10 Hz, and that the landing response at high contrast has a higher optimum of $\approx$ 20 Hz. Such differences indicate the involvement of different neuronal mechanisms.

Responses to movement presented in a field with the center frontally, thus including the binocular overlap of the visual fields of both eye (≈ 5 - 10°, Beersma et al. 1977), are complicated. The main direction of sensitivity is the upward direction (Taddei and Fernandez 1973; Eckert 1980; Wehrhahn et al. 1981; Eckert and Hamdorf 1983), but also horizontal movement is very effective in eliciting the landing response, as follows from experiments with two vertical stripes moving apart (e.g. Eckert et al. 1979). The movement sensitivity in the frontal eye region has been tested in experiments on monocular flies (one eye covered by black paint). The uncovered eye was stimulated by the combination of an excitatory, front to back, horizontal movement in the monocular lateral part of the visual field and different directions of movement presented in the frontal part of the visual field. In these experiments Wehrhahn et al. (1981) have shown that horizontal movement to the left presented in the frontal equatorial region of the right eye contributes in an excitatory manner to eliciting the landing response.

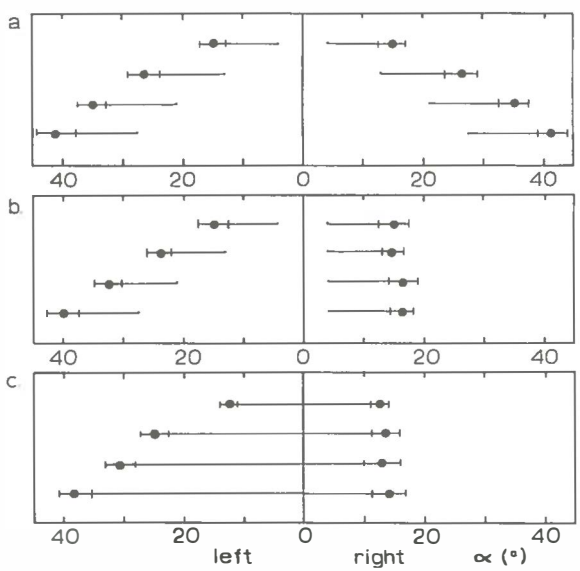


Fig. 14. Horizontally outward moving dark stripes of width 3.5° generated on a display screen, at 10 s intervals, moving (at 150°/s) from different start positions to 45° laterally. α indicates the angular position, . stripe position at the moment of the response. Lines indicate angular displacements of the stripes in the interval between the start of the movement and the response. a.symmetrically moving stripes b.asymmetrically moving stripes c. time delayed movement of the right stripe.

Similar results have been obtained by Eckert and Hamdorf (1983). This main direction of sensitivity of the frontal visual field is probably located in the contralateral part of binocular visual field and is opposite to that of the lateral, monocular, visual field.

In several respects, striking differences seem to exist in the responses to periodic spatial patterns and those to two single stripes moving apart. For instance, near the median plane the sensitivity to upward movement of periodic patterns is high above the equator and the sensitivity to downward movement of periodic patterns is high below the equator. When two single horizontal stripes, moving apart, combine these two directions of movement in one pattern, i.e. upward movement in the upper part of the visual field and downward movement in the lower part, the striking result is no response at all (Eckert 1980,1983).

Furthermore, at horizontal outward movement, presented to the equatorial monocular parts of both eyes, the responsivity to the periodic stripe pattern is high (Wehrhahn et al. 1981; Borst 1986; Borst and Bahde 1986,1987). With two outward moving single vertical stripes, one at either side of the median plane, Eckert et al. (1979; Eckert and Hamdorf 1980) have demonstrated that the sensitivity to horizontal movement is high near the median plane, but decreases rapidly just outside the binocular visual field.

We have tested horizontal movement sensitivity, and used a display screen which generated a pattern consisting of two vertical dark stripes in a bright field. At intervals, the stripes moved apart with constant average angular velocity, 150°/s. The velocity was higher than the 40°/s in the experiments by Eckert. Fig. 14a shows that the angular displacement of the stripes, after which the response is detected, does hardly depend on the start position of the stripes. In Fig. 14b, where the start position of the stripe is frontally in the right half of the visual field and variable in the left half, similarly, the angular displacement of the stripes, after which the response is detected, did hardly depend on the start position also. In Fig 14c the movement of the right stripe was presented delayed with respect to the movement of the left stripe (i.e. each of the stripes initially appeared at the mid-line of the screen and subsequently moved outward). The responses occurred after sufficient movement of both stripes, as is indicated in the figure. In these experiments the movement of exclusively one stripe did not elicit the landing response at all. The results show that the movement sensitivity was approximately equal in the parts of the visual field tested and suggest that the special sensitivity for horizontal outward movement in the binocular visual field is more important at lower angular velocity than at higher angular velocity.

On the display screen, instead of the two vertical stripes, was generated

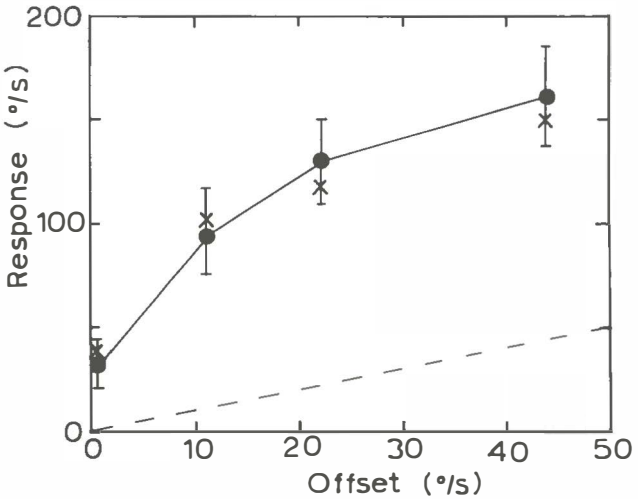


Fig. 15. Angular velocity threshold values, horizontal outward movement. Periodic stripe pattern of spatial wavelength 14° in a 90° x 50° field with the center below the equator. Offset: angular velocity continuously presented.

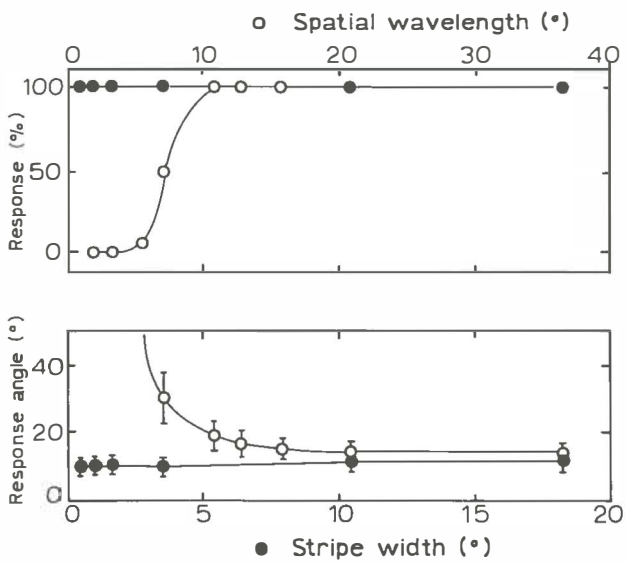


Fig. 16. Response angle and response percentage to horizontal outward movement of dark stripes and periodic stripe patterns, 75°/s.

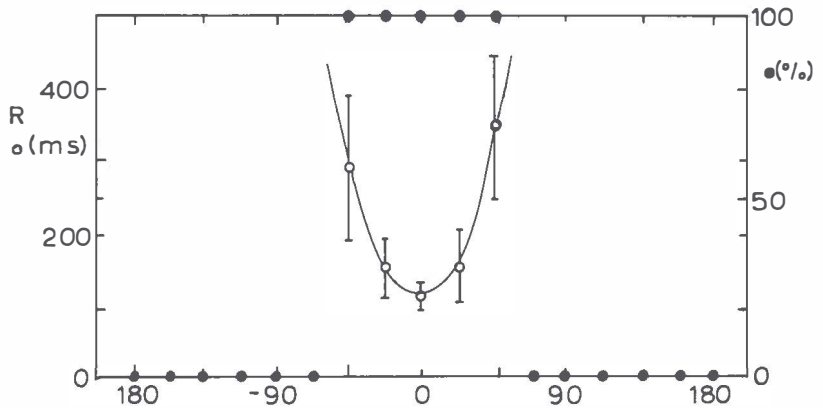


Fig. 17. Response latency and response percentage. Movement in opposite directions at either side of the visual field, 75°/s . o = horizontal outward. Fields 40° laterally, just below the equator.

a pattern consisting of two periodic stripe patterns. The threshold angular velocity of the movement was determined. The angular velocity was gradually increased until the animal responded (in ≈ 5 s). Then the movement stopped. After 10 s the animal was retested. The thus obtained threshold angular velocity was ≈ 35°. In similar tests, continuous movement was presented during the 10 s intervals. Due to this movement the threshold angular velocity shifts to higher values (Fig. 15). To the landing response, these results indicate that adaptation and habituation can play a substantial role.

At angular velocities of the stripes higher than 35°/s a response was elicited in ≈ 100 % of the tests. Although the response percentages were identical, the response moments to various higher angular velocities were not identical. At movement of angular velocity 75°/s, the average response latency was as short as 130 ms, and, at 150°/s 90 ms, at 300°/s 70 ms, and at 600°/s ≈ 60 ms. Especially with respect to the highest velocity it has to be noted that the angular velocity was in fact the average angular velocity of a stepwise movement of the stripes. The latency values are similar to those measured by Borst and Bahde.

The dependence on the stripe width and spatial wavelength was tested, using two dark stripes moving apart and two periodic stripe patterns moving apart. The results are presented in Fig. 16. The stripes did elicit the landing response, independent the stripe width. The periodic stripe patterns elicited responses for larger spatial wavelengths.

Finally, with a pattern, presented in a circular field and moving in different directions, is tested the directional selectivity of the movement

sensitivity. Because the angular velocity is rather low the distribution is narrow (Fig 17). The data show that the horizontal outward direction is the main direction of sensitivity in the area stimulated, 40° laterally and just below the equator.

All experiments performed so far demonstrate that outward movement is the adequate stimulus for the landing response. This is probably also the case with approaching objects. The change in distance does not directly contribute. Especially those stimuli, which contain two outward movement components at the same time, have demonstrated high efficacy in eliciting the landing response. Low angular velocities and small spatial wavelengths do hardly contribute.

Flying flies generally avoid suddenly appearing obstacles by executing a course correction in the opposite direction. In the case of asymmetrical stimulation, e.g. by an approaching object movement from one side, the direction in which the correction has to be executed for a successful avoidance is obvious. At symmetrical stimulation execution of a landing response might be a better solution to avoid crashes.

References

Beersma DGM, Stavenga DG, Kuiper JW (1977) Retinal latice, visual field and binocularities in flies. J Comp Physiol 102: 305-320

Borst A (1986) Time course of the houseflies' landing response. Biol Cybern 54: 379-383

Borst A, Bahde S (1986) What kind of movement detector is triggering the landing response of the housefly? Biol Cybern 55: 59-69

Borst A, Bahde S (1987) Comparison between the movement detection system underlying the optomotor and the landing response in the housefly. Biol Cybern 56: 217-224

Braitenberg V, Taddei Ferretti C (1966) Landing reaction of *Musca domestica* induced by visual stimuli. Naturwissenschaften 6: 155-156

Burkhardt D, Darmhofer-Demar B, Fischer K (1973) Zum binokularen Entfernungssehen der Synsekten. J Comp Physiol 87: 165-188

Eckert H (1980) Orientation sensitivity of the visual movement detection system activating the landing response of the blowflies, *Calliphora* and *Phaenicia*: a behavioural investigation. Biol Cybern 37: 235-247

Eckert H (1982) Radial pattern expansion drives the landing response of the blowfly, *Calliphora*. Naturwissenschaften 69: 348-349

Eckert H (1983) On the landing response of the blowfly, *Calliphora erythrocephala*. Biol Cybern 47: 119-130

Eckert H, Hamdorf K (1980) Excitatory and inhibitory response

components in the landing response of the blowfly, *Calliphora erythrocephala*. J Comp Physiol 138: 253-264

Eckert H, Hamdorf K (1981) The contrast frequency dependence: a criterion for judging the non-participation of neurones in the control of behavioural responses. J Comp Physiol 145: 241-247

Eckert H, Hamdorf K (1983) Does a homogeneous population of elementary movement detectors activate the landing response of b owflies, *Calliphora erythrocephala*? Biol Cybern 48: 11-18

Eckert H, Fligge B, Hamdorf K Excitation and inhibition of the landing response of the blowfly, *Calliphora*. Naturwissenschaften 66: 368-370

Fernandez Perez de Talens A, Taddei Ferretti C (1970) Landing reaction of *Musca domestica*: dependence on dimension and position of the stimulus. J Exp Biol 52: 233-256

Fernandez Perez de Talens A, Taddei Ferretti C (1975) Landing and optomotor responses of the fly *Musca*. In: Horridge GA (ed) The compound eye and vision in insects. pp 490-501 Clarendon Press, Oxford

Fischbach KF (1981) Habituation and sensitization of the landing response of *Drosophila melanogaster*. Naturwissenschaften 68: 332

Franceschini N, Kirschfeld K (1971) Etude optique *in vivo* des éléments photorécepteurs dans l'oeil composé de *Drosophila*. Kybernetik 8: 1-13

Goodman LJ (1960) The landing response of insects. I. The landing response of the fly *Lucilia sericata* and other Calliphorinae. J Exp Biol 37: 845-878

Goodman LJ (1964) The landing response of insects. II. The electrical response of the compound eye of the fly *Lucilia sericata* upon stimulation by moving objects and slow changes in light intensity. J Exp Biol 41: 403-415

Taddei Ferretti C, Fernandez Perez de Talens A (1973a) Landing reaction of *Musca domestica*, III: dependence on the luminous characteristics of the stimulus. Z Naturforsch 28c: 568-578

Taddei Ferretti C, Fernandez Perez de Talens A (1973b) Landing reaction of *Musca domestica*, IV: A. Monocular and binocular vision. B. Relationship between landing and optomotor reaction. Z Naturforsch 28c: 579-592

Taddei Ferretti C, Fernandez Perez de Talens A (1975) The effect of illumination on the landing response. In: Horridge GA (ed) The compound eye and vision in insects. pp 502-512 Clarendon Press, Oxford

Wagner H (1982) Flow-field variables trigger landing in flies. Nature 297: 147-148

Wehrhahn C, Hausen K, Zanker J (1981) Is the landing response of the housefly (*Musca*) driven by motion of a flow field? Biol Cybern 41: 91-99

Chapter VI

HORIZONTAL MOVEMENT SENSITIVITY OF THE LANDING RESPONSE IN THE HOUSEFLY.

Abstract

Landing responses elicited by visual stimuli have been measured on stationary flying houseflies, *Musca domestica*. Pattern speed dependence of horizontal movement sensitivity was investigated by applying stepwise outward movement of vertical stripes, presented in the medio-frontal visual field of the fly. The two dark stripes, one on either side of the bright stimulus field, moved in smaller or larger steps (Fig. 1). Both step angle and step interval of the movement stimulus were varied; their combination determined the average angular velocity of the movement. The response, being a constant phase in the transition from flight to execution of the landing response, was optically measured from fore leg movement.

Repeated presentation of identical test stimuli resulted in responses having a probability distribution function with a steep slope and a tail toward longer response times, indicating that the response is not entirely normally distributed.

The response moments did depend on the average angular velocity of the movement; the time span between stimulus onset and the response decreased with increasing pattern velocity. The decrease was less than proportional and the corresponding angular displacement of the stripes increased less than proportional to the pattern velocity.

The sensitivity to the movement of the two stripes was high. The responses were executed very rapidly (Fig. 2). The most rapid responses were obtained at average angular velocity 600°/s (movement in steps of $\delta a = 3.5°$ and $\delta t = 5.8$ ms) where the 50 % response was detected within 45 ms after the onset of the movement.

Stimulus and response are separated by a delay, lasting on the average 30 ±5 ms. Responses did not occur later than ≈ 40 ms after the termination of the movement. The delay and the response moments together suggest that movement over an angle covering 3 - 5 neighbouring visual axes is sufficient to elicit the landing response under conditions that the movement is presented in small steps. With 3 - 5 successive movement steps, each with an angular distance larger than the interommatidial angle, the movement is sufficient to elicit the landing

response also. The responses then occur 25 -35 ms later, and are nearly independent of the average angular velocity of the movement.

The responses to movements in steps of different size (angles of 0.45° - 7°) demonstrate that interactions between neighbouring neuro-ommatidia, as well as interactions between neuro-ommatidia with visual axes separated by angles larger than the interommatidial angle, contribute to perception of movement and thus can elicit the landing response.

Introduction

Since the first detailed experiments of Goodman (1960, 1964) the landing response of flying insects has been extensively studied, especially on dipterans. The rather complex behavioural response, which basically appears as a fixed action pattern, is executed at attempts to terminate flight. At least three different response components can be distinguished: a decelerating action of the wings, a change in the orientation of the body, and changes in the position of the legs. However, in a successful landing often additional substrate oriented response components can be distinguished. Therefore, the landing response is interpreted to be the response pattern produced by the animal at attempts to perform a landing in a standard manner in order to avoid imminent crashes into suddenly appearing obstacles, especially under conditions that the alternative of a course correction seems not to be present. Then the unfolded legs provide the opportunity of a final deceleration at contact with the substrate.

Among the stimuli eliciting the landing response visual stimuli are very prominent, and consequently the occurrence of the landing response has been used to investigate characteristics of the visual system. Most experiments have been performed on tethered, stationary flying animals (e.g. Braitenberg and Taddei 1966; Fernandez and Taddei 1970,1975; Taddei and Fernandez 1973a,b,1975; Coggshall 1972; Eckert et al. 1979; Eckert 1980,1982,1983; Eckert and Hamdorf 1980,1981,1983; Fischbach 1981; Wehrhahn et al. 1981; Tinbergen and Abeln 1983; Borst 1986; Borst and Bahde 1986,1987). In stationary flying animals execution of the landing response is detected from leg movements. The visual characteristics of the prelanding flight behaviour have been investigated on free flying animals (Wagner 1982). In the present paper the occurrence of the landing response in stationary flying houseflies *Musca* has been tested to investigate the sensitivity to horizontal movement.

During flight the legs of the fly are held in a special position related with the streamline of the body. Generally, the front legs are folded in the space between head and thorax with the tarsi pointing forward just below the head, the middle legs folded between thorax and abdomen with the tarsi below the thorax and the hind legs pointing backward besides

the abdomen. This posture is encountered in many dipterans including *Musca* (Goodman 1960; Borst 1986). In some species a slightly different position of the legs is observed, for instance in *Drosophila* the fore and middle legs are bent backward and held close to the body during flight. Upon execution of the landing response the legs suddenly extend. The fore legs move sideward and upward with a final position of the tarsi just above and beside the head, the middle legs unfold with mainly downward and sideward movement and in many species the hind legs also move sideward and downward. According to a detailed analysis of the leg movements in *Musca* the unfolding of the fore legs takes 30 - 40 ms, whereas the hind legs hardly take part in the response (Borst 1986).

Visual stimuli, which play a primary role in eliciting the landing response, show that the reaction is produced to moving stimuli and, furthermore, to changes in intensity. Adequate moving stimuli are simple objects approaching the stationary flying animal (Goodman 1960; Eckert and Hamdorf 1980). Patterns moving at a fixed distance in respect to the animal are effective when they contain movement in outward (progressive) or expanding directions and therefore have aspects in common with an approaching object. Among these stimuli periodic stripe patterns and sinewave patterns are effective depending on contrast, spatial wavelength, spectral wavelength, direction and angular velocity of the movement and on the part of visual field which is stimulated (Fernandez and Taddei 1970,1975; Taddei and Fernandez 1973a,b; Coggshall 1972; Eckert 1980,1982,1983; Eckert and Hamdorf 1981,1983; Wehrhahn et al. 1981; Tinbergen and Abeln 1983; Borst 1986; Borst and Bahde 1986,1987). Furthermore, the movement of single stripes or contrast borders is generally very effective also (Goodman 1960; Eckert et al. 1979; Eckert 1980; Fischbach 1981). Inhibitory effects can be obtained from movement in the opposite direction (Eckert et al. 1979; Eckert and Hamdorf 1980; Wehrhahn et al. 1981).

The main problem underlying the present study is: how much movement c.q. how many movement events are necessary to induce the landing response. Therefore the horizontal movement sensitivity of the neural pathway involved in the landing response has been investigated by applying stepwise lateral expanding movement of two vertical stipes. Different average angular velocities of the movement were obtained by keeping the duration of the interval between the successive movement steps constant, whereas the step angle was varied. In a further series of experiments both step interval and step angle were varied. Responses occurred in a wide range of average angular velocities of the movement. The data are discussed from the view point that a limited number of movement events is sufficient to elicit the landing response under quite different movement conditions.

Methods

The experiments were performed on 5 - 20 days old female houseflies *Musca domestica* (wild type) reared in the laboratory.

A V-shaped piece of aluminum wire was connected to the dorsal side of the thorax and the head. Particular attention was paid to that the compound eyes were uncovered. The animals, with the wire on the back, were kept in cages and supplied with sugar and water.

The experimental animal was attached with the aluminum wire to a goniometer located in front of a display screen. Optical alignment to the display symmetry axis was obtained by using a swing-in epi-illumination microscope. The animal was positioned in such a way that the equatorial deep pseudopupil (Franceschini and Kirschfeld 1971) in both compound eyes was seen in the center of the microscope.

An optical leg-movement detector unit, consisting of a narrow beam of light directed to a photodiode, was mounted just beside the head of the fly. The beam was oriented vertically, 20° tilted and placed close to the compound eye, so allowing detection of predominantly the sideward component of the movement of the fore leg and preventing interruption of the beam by the flapping wing.

The visual stimulus consisted of a moving stripe pattern, generated on the display screen (Hewlett Packard 1311A, phosphor P31). The repetition frequency of the display was 173 Hz, i.e. the pattern was refreshed each 5.8 ms. The stimulus pattern, presented in a 85° x 55° field at a distance of 11 cm from the fly, was basically built up from 240 vertical lines which each could be set bright green ("on") or dark ("off"). The angle between neighbouring lines was 0.45° in the center of the field and slightly smaller in the periphery. The spatial pattern configuration used in the experiments consisted of two vertical dark stripes in a bright field (Fig. 1). The width of the dark stripes was 3.5° (8 lines) and their initial spatial separation 9°. The stripes were always symmetrical with respect to the vertical mid-line of the display and the visual field of the fly. The intensity of the bright field was 60 mW/(m^2sr), that of the dark stripes approximately 12 mW/(m^2sr).

In a test experiment, at t = 0 the stripes moved horizontally outward, i.e. the left stripe moved to the left and the right stripe moved to the right (Fig. 1). Generally the movement continued until the stripes disappeared on either side of the display in the periphery of the stimulus field, thus leaving an entire bright field. Subsequently, after a delay, the stripes were reset to the initial position. In part of the experiments (e.g. Fig. 4) the movement was stopped at an intermediate stripe position, i.e. before the stripes reached the display boundary.

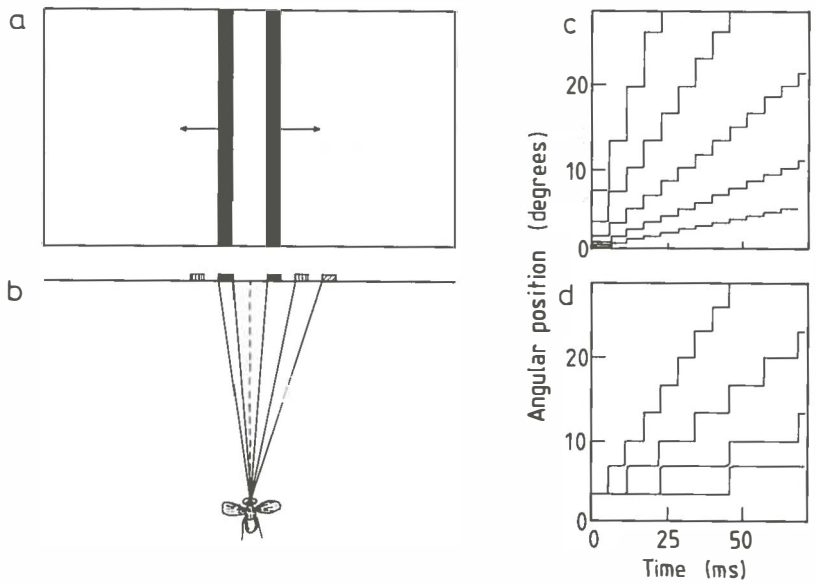


Fig. 1. Diagram of the stimulus set-up. The stimulus pattern is built up from 240 neighbouring lines generated on a HP 1311A P31 display screen and consists of two dark stripes of 3.5° width (8 lines) in a bright green field of 85° x 55° placed at a distance of 11 cm from the stationary flying fly. Frame frequency of the pattern generation was 173 Hz (5.8 ms / frame). Both stripes moved outward due to successive displacement of the stripes in steps of N lines at intervals of M frames of the pattern generation. With new frames every 5.8 ms and the angle between neighbouring lines 0.45° the average angular velocity of the movement w amounts to w = (N x 0.45 x 173 / M) °/s, with M and N being integers. a. The two vertical dark stripes on the screen at start position, each 4.5° laterally from the symmetry line. Arrows indicate outward direction of the stepwise movement of the stripes. b. The pattern as presented to the medio-frontal visual field of the fly. Left of the symmetry line the stripe of 3.5° width is indicated at the start position and after a movement step over 7°; right of the symmetry line two successive stripe positions (hatched) after movement in steps over 7° are indicated. Inner edge of the stripe positions 4.5°, 11.5° and 18.5° from the symmetry line, respectively. c. Successive angular positions of the stripes at stepwise movement with constant step interval δt = 5.8 ms and different step angles δa = 0.45, 0.9, 1.8, 3.5 and 7°. Average angular velocities of the movement w = 75, 150, 300, 600 and 1200°/s (upper trace). d. Successive angular positions of the stripes at stepwise movement with constant step angle δa = 3.5° and different step intervals δt = 5.8, 11.6, 23.2 and 46.4 ms, resulting in average angular velocities w = 600, 300, 150 and 75°/s (lower trace).

The movement was realized by repetitively shifting the stripes stepwise over an angle δa with a time interval δt. The resulting average angular velocity of the movement was $w = \delta a / \delta t$. The step angle δa was equal to or a multiple of the width of the vertical lines of the display: $\delta a = N \times 0.45°$. The time interval δt was equal to or a multiple of the time span of pattern generation: $\delta t = M \times 5.8 \times 10^{-3}$ s. With M = 1 (an interval of 5.8 ms) and N = 1, 2, 4, 8, 16 and 32, i.e. steps of 0.45, 0.9, 1.8, 3.5, 7 and 14° the average angular velocity was w = 75, 150, 300, 600, 1200 and 2400°/s, respectively. The velocity w = 37.5°/s was realized by taking M = 2 (δt = 11.6 ms) and N = 1 (δa = 0.45°); see Fig. 2. Average angular velocities w = 75, 150, 300, 600 and 1200°/s were obtained also by combining N = 8 and 16 with M = 1, 2, 4 and 8 (Fig. 5 and 6).

For flight stability both a slight airstream, directed to the antennae, and a low background intensity was presented during all experiments. Under these conditions the animals folded the fore and middle legs, lifted the hind legs and performed stationary flight.

The stimulus pattern on the screen in front of the fly was kept stationary for 5 s. Then the stripes moved stepwise from the center to the periphery of the stimulus field. Interruption of the detector beam indicated a response of the animal and the moment of the response relative to the movement of the pattern and was registered using an electronic counter with gated clock signal. No actual landing substratum was presented.

Subsequently, the stripes were reset to the initial position. The animal folded the legs again, as was visually controlled by the experimenter. After an adaptation period, lasting again 5 s the animal was retested, using the same or different parameter settings. The experiments lasted several hours. At regular intervals the animal was allowed to stop flight and drink sugar water.

Results

Responses to stepwise movement with different step angles.

The time intervals between movement onset and the occurrence of the landing response are presented in the cumulative histograms of Fig. 2. At average angular velocities w = 150, 300 and 600°/s a 100 % response was obtained. Responses were measured in part of the tests only for both low and high average angular velocities: w = 37.5, 75 and 1200°/s. At w = 2400°/s (not shown) never a response could be elicited. The responsivities are given in Table I. The data of Fig. 2 also show that responses to identical test stimuli occur at quite variable moments after

movement onset. Furthermore, the distribution of the response moments distinctly depends on the value of the average angular velocity w. This dependence is demonstrated in the change of T_{50}, i.e. the time span within which a response had occurred in 50 % of the tests; see Table I.

The probability distribution functions of Fig. 2 show an increasingly steeper slope at increasing pattern speed w. Therefore, the data are also presented as a function of the angular displacement of the stripes, i.e. the angle between the initial stripe position and the stripe position at the moment that the obtained percentage of the tests had resulted in a landing response (Fig. 3). Clearly, at those average angular velocities where the

Table I.

δa (°)	δt (ms)	w (°/s)	R_C (%)	T_{50} (ms)	S_{50} (step)	A_{50} (°)	S'_{50} (step)	A'_{50} (°)
0.45	11.6	37.5	10	-	-	-	-	-
0.45	5.8	75	80	140	24	10.5	19	8.2
0.9	5.8	150	100	75	13	11.5	8	7.2
1.8	5.8	300	100	60	11	19	6	10.5
3.5	5.8	600	100	45	8	27	3	10.5
7	5.8	1200	85	55	9	>38	4	30
14	5.8	2400	0	-	-	-	-	

δa = *step angle; angle between successive stripe positions.*
δt = *step interval; time interval between successive stripe positions.*
w = *average angular velocity of the movement determined by* δa *and* δt.
R_C = *cumulative responsivity.*
T_{50} = *time span within which a response has occurred in 50 % of the tests.*
S_{50} = *number of movement steps corresponding to* T_{50}.
A_{50} = *angular displacement of the stripes corresponding to* T_{50}.
$T_{50} \cdot w = A_{50} = S_{50} \cdot \delta a$
S'_{50} = *number of movement steps triggering a response at* T_{50} *taking into account the delay (see text).*
A'_{50} = *angular displacement of the stripes triggering a response at* T_{50} *taking into account the delay (see text).*

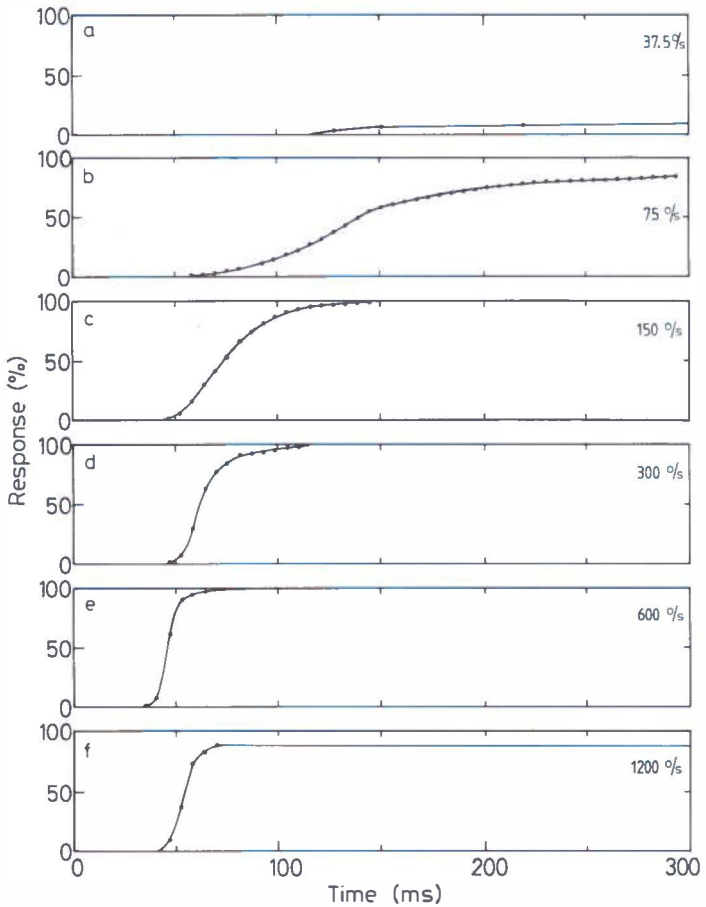


Fig. 2. Cumulative histograms of landing response moments. Results obtained by optical detection of fore leg movement at stimulation by stepwise horizontal movement of two vertical stripes (width 3.5°). The stripes started their outward movement at 4.5° laterally and finally disappeared in the periphery of the 85° field. The different average angular velocities of the movement were obtained by changing the step angle (δa = 0.45° - 7°), whereas the step interval was kept constant (δt = 5.8 ms), except in a. where a step interval δt = 11.6 ms was used. Average angular velocities of the movement were as indicated. Data based on 1800 responses in 2 female flies.

responsivity is 100 %, i.e. w = 150, 300 and 600°/s, the probability distribution functions are virtually identical, except for a relative angular shift of 7 - 8°. These distribution functions show a steep slope and a tail toward larger angles, indicating that the distribution is not entirely normal. The A_{50} values, the angular displacement of the stripes corresponding to T_{50}, are given in Table I.

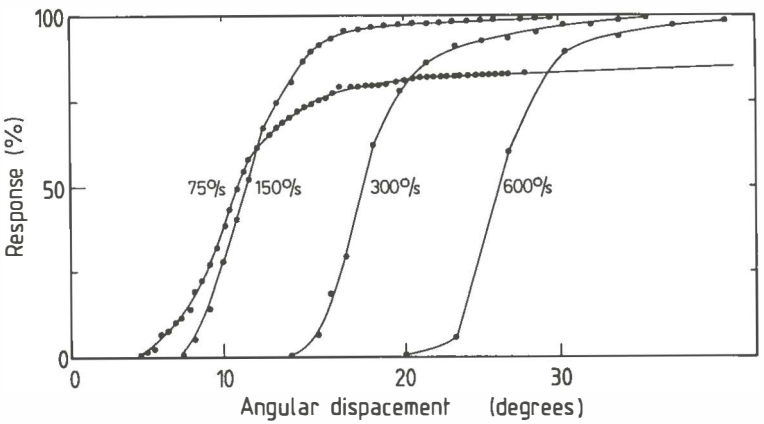


Fig.3. Cumulative histograms of the landing responses as function of the angular displacement of the two vertical stripes (width 3.5°) moving stepwise outward at constant average angular velocity, from 4.5° laterally to the periphery of the 85° stimulus field. The different average angular velocities of the movement indicated in the graph were obtained by changing the step angle (δa = 0.45° - 3.5°): the step interval was kept constant (δt = 5.8 ms). Data based on 1400 responses in 2 animals.

Response delay.

In the case of stimulation with the pattern moving at average angular velocity w = 1200°/s, responses occurred after the stripes had already disappeared from the screen. In order to illustrate this Fig. 2f has been redrawn in Fig. 4a where the moment of disappearance of the stripes is indicated by END. The response distribution of Fig. 4b demonstrates that the responses did occur between 5 and 35 ms after the end of the movement stimulus, and therefore a delay between stimulus and response is likely. This was also investigated with lower pattern speed. An example is presented in Fig. 4c,d, where the average angular velocity was w = 150°/s, but this time with a stop of the movement after 65 ms, i.e.

with the stripes at an intermediate position ≈ 10° from the start position. Responses then occurred in 45 % of the tests. Virtually all of these responses did occur after the termination of the movement stimulus, and within ≈ 40 ms. These results indicate that the delay between stimulus and response does not exceed 40 ms. The average duration of the delay was

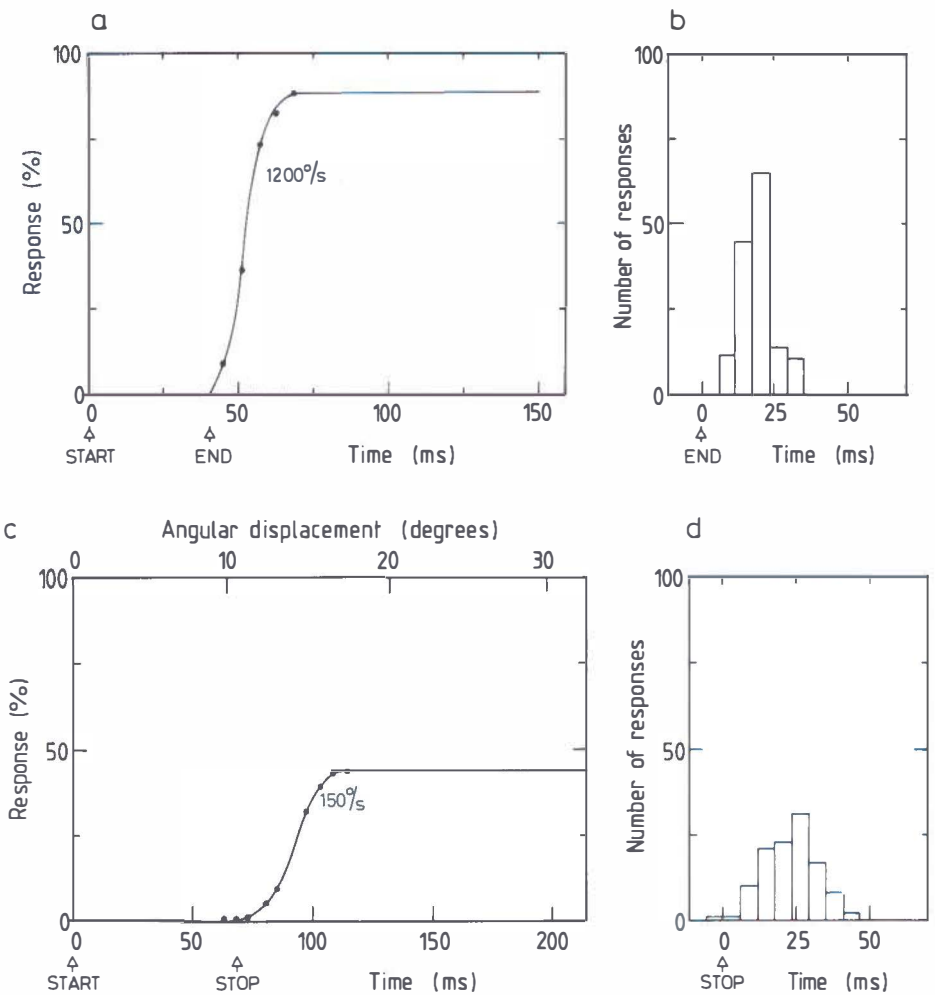


Fig. 4. Response delay. Cumulative histograms and histograms of landing response moments. a, b. Average angular velocity of the movement w = 1200°/s. END indicates the moment at which the stripes disappeared in the periphery of the stimulus field. c, d. Average angular velocity of the movement 150°/s. Stop indicates the intermediate stop of the stripe movement.

estimated at 30 ± 5 ms in tests where patterns with average angular velocities ranging from w = 75 to 1200 °/s were applied. Because the interval time in these patterns was δt = 5.8 ms, the delay time of 30 ± 5 ms corresponds to 5 ± 1 movement steps. Consequently, in the tests of Fig. 2b-f, the last 4 - 6 movement steps before execution of the landing response did not contribute to the release of the response. Furthermore, a 30 ms delay implies that 50 % of the responses are actually triggered within a time span T_{50} - 30 ms. When w = 150 °/s, for example, T_{50} - 30 ≈ 45 ms, see Table I. This period of 45 ms corresponds to a movement of ≈ 8 steps and an angular displacement of the stripes of $A'_{50} = (T_{50} - 30) \times w \approx 7°$. Because the horizontal interommatidial angle in the compound eye of *Musca* is ≈ 2 ° (Beersma et al. 1975) movement over 7 ° corresponds to movement over 3 - 4 columns of visual axes. For the average angular velocities w = 75 to 300 °/s the T_{50} - 30 values correspond to movement over angles A'_{50} of ≈ 7 ° to 10 °, i.e. over 3 - 5 columns of visual axes. Or, at these average angular velocities the successive stimulation of 3 - 5 neighbouring columns of visual axes is sufficient to trigger the landing response in 50 % of the tests.

For the higher average angular velocities, w = 600 °/s and 1200 °/s, the step angles (δa = 3.5 ° and 7 °, Table I) are larger than the interommatidial angle. The T_{50} -30 values then correspond to 3 - 5 movement steps over an angle of 3.5 ° or 7 ° each, which appear to trigger the landing response in 50 % of the tests. Note that the efficiency of triggering responses diminishes when δa = 7 °.

Responses to stepwise movement with different step intervals.

In a further series of experiments special attention was paid to the interval of the steps. The step angle was kept constant (δa = 3.5 or 7 °) and the step interval was varied in order to test the dependence on longer step intervals. In the movement presented first the step angle was δa = 3.5 ° and the step interval δt = 5.8, 11.6, 23.2 and 46.4 ms, resulting in average angular velocities w = 600, 300, 150 and 75 °/s, respectively. The results are presented in Figs 5a, 6a and Table II. A responsivity of 100 % was reached, except when the interval was 46 ms (w = 75 °/s) where no response was obtained. In Fig. 5a the cumulative response moments are presented. The corresponding T_{50} values are indicated in Table II. In Fig. 6a the cumulative responses are plotted as a function of the number of the successive movement steps. The data show that the responses occurred after a small number of movement steps. This number of steps decreases with increasing duration of step interval (with decreasing average angular velocity). Again, when a 25 - 35 ms delay

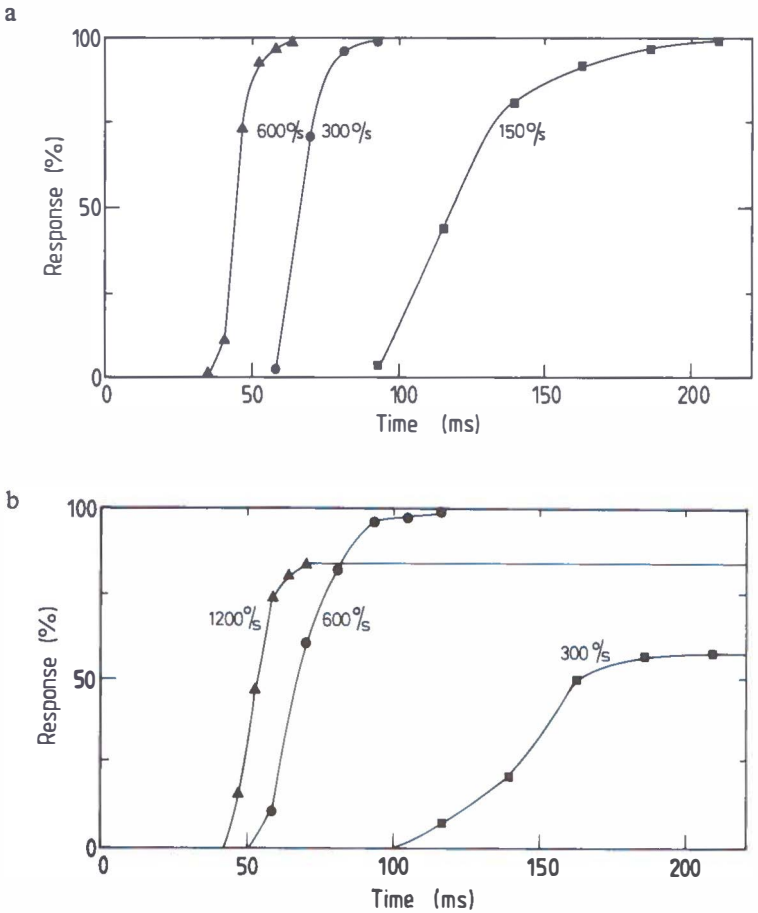


Fig. 5. Cumulative histograms of the landing response moments. Stepwise movement having different step intervals (δt 5.8 ms, 11.6 and 23.2 ms). a. Step angle δa = 3.5°. b. Step angle δa = 7°. triangles: δt = 5.8 ms, dots: δt = 11.6 ms, squares: δt = 23.2 ms. Average angular velocities of the movement indicated in the graphs.

between stimulus and response is taken into account, only 3 - 4 movement steps had occurred preceding the delay and the 50 % response. Because this was the case at all these three pattern speeds it seemed that the number of relevant movement steps was independent of the step interval and thus independent of the average angular velocity of the movement.

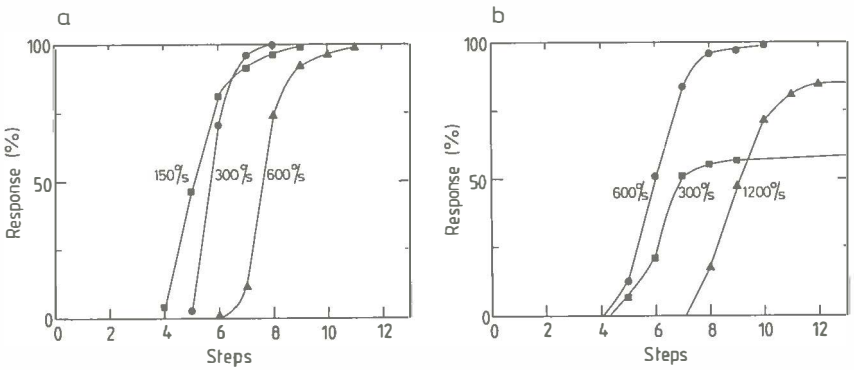


Fig. 6. Cumulative histograms of the landing responses as a function of the number of movement steps made by the pattern. Different step intervals δt = 5.8, 11.6 and 23.2 ms. a. Step angle δa = 3.5° b. Step angle δa = 7°. Triangles: δt = 5.8 ms, dots: δt = 11.6 ms, squares: δt = 23.2 ms.

Table II.

δa (°)	δt (ms)	w (°/s)	R_C (%)	T_{50} (ms)	S_{50} (step)
3.5	5.8	600	100	45	8
3.5	11.6	300	100	70	6
3.5	23.2	150	100	115	5
3.5	46.4	75	0	-	-

A slightly different situation occurred at stimulation with the stepwise movement composed of 7° step angles and variable step interval δt = 5.8, 11.6, 23.2 and 46.4 ms, i.e. in average angular velocities w = 1200, 600, 300 and 150°/s. The results are presented in Figs 5b, 6b and Table III. Again no response was measured when movement with the step interval of 46.4 ms (w = 150°/s) was presented. This suggests that the interval duration was too long to obtain responses at all.

As shown in the figures the movement with the step interval of 11.6 ms (w = 600°/s) resulted in a responsivity of 100 %, whereas with both 5.8 ms and 23.2 ms intervals (w = 1200 and 300°/s) the response percentage was reduced, see Table III. At δt = 11.6 ms (w = 600°/s) the

Table III.

δa (°)	δt (ms)	w (°/s)	R_C (%)	T_{50} (ms)	S_{50} (step)
7.0	5.8	1200	85	55	9
7.0	11.6	600	100	70	6
7.0	23.2	300	60	160	7
7.0	46.4	150	0	-	-

50 % response was reached after $S_{50} \approx 6$ steps (Fig. 6b). By taking into account a 25 - 35 ms delay, again, it follows that 3 - 4 movement steps had occurred before the delay and the 50 % response. This was not the case with both the longer and shorter intervals (lower and higher average angular velocities) where a few more steps (1 - 2 additional steps) were found.

Discussion

The response.

Upon visual stimulation with horizontal movement by stepwise outward moving stripes the tethered stationary flying houseflies *Musca* respond with movement of the legs. As explained in the introduction this behavioural action is regarded as a component of execution of the landing response (Goodman 1960).

Borst (1986) has measured the time course of the vertical component of the leg movements of the landing response in *Musca*. According to his results the movement of the fore leg takes 30 - 40 ms, from the start of the movement with the tarsi below the head to the final position with the tarsi above the head. Because in the experiments presented here the detector beam was located very close to the fore leg position during flight, and therefore the moment of activation of the detector corresponds to the start of the fore leg movement within a few ms and is virtually identical to the start of upward fore leg movement in the experiments of Borst (1986).

In the method of optical detection used by Borst and Bahde (1986, 1987) a different phase of the fore leg movement is measured due to a different position and orientation of the detector beam.

Response distributions.

The cumulative histograms of the responses to repeated identical stimulation have been depicted as a function of the angular displacement of the moving stripes. The response distribution curves are similar in shape and show an increasing angular shift indicating a larger angular displacement at higher pattern speed. This correspondence in shape, especially in the 4 curves of Fig. 3, demonstrates a strong dependence on the angular displacement of the stripes. Responses to identical test stimuli were not entirely normally distributed. The skewness of the distribution curves indicates a relative excess of responses occurring after a larger angular displacement of the stripes.

Movement sensitivity.

Horizontal outward movement of two vertical stripes, mechanically or electronically driven, is an effective stimulus for eliciting the landing response (Eckert et al. 1979; Eckert 1980; Eckert and Hamdorf 1980; Fischbach 1981). In the present experiments on *Musca* always a response was measured when the stripes moved at average angular velocities in the range between 75°/s and 1200°/s. These values are considerably higher than those obtained by Eckert in experiments on *Calliphora* stimulated by mechanically moving stripes. In these experiments the response induced by stripes stepping over angles of 4.6° already saturates at an average angular velocity as low as 4°/s (corresponding with a step interval $\approx$ 1 s); see Eckert et al. (1979), Eckert (1980), Eckert and Hamdorf (1980). However, this difference in sensitivity is probably not only the consequence of the difference in species studied, but might depend on the properties of the mechanical vs. electronic method of stimulation also. In the experiments presented here the average angular velocity of 37.5°/s was the lowest pattern speed at which the landing response was elicited in *Musca* and the responsivity was small. Interestingly, at this average angular velocity, created by step angle $\delta a = 0.45°$ and interval $\delta t = 11.6$ ms, neighbouring photoreceptors, separated by a horizontal interommatidial angle of $\approx 2°$ (Beersma et al. 1975) are successively stimulated after $2°/0.45° \approx 4.4$ movement steps, i.e. at intervals of $\approx$ 50 ms. In the tests with the larger step intervals ($\delta a = 3.5°$ and 7°, w = 75°/s and 150°/s) a step intervals of 46.4 ms was too long for obtaining responses at all. Landing responses were elicited in *Musca* by

movement with intervals shorter than ≈ 50 ms, values more than an order of magnitude smaller than the 1 s mentioned above. The average angular velocities at which the responses were elicited by the two stripes were similar to the pattern speeds at which periodic patterns (sinewave patterns and stripe patterns) did elicit the landing response (e.g. Eckert 1980; Eckert and Hamdorf 1981; Wehrhahn et al. 1981; Borst 1986; Borst and Bahde 1986,1987).

Response moments.

The moments at which the landing responses were detected thus yielded the durations of the movement stimulus before the response. The timespan within which a response has occurred in 50 % of the tests, T_{50}, ranged from 45 - 140 ms in the test series with constant step interval (δt = 5.8 ms) and the varying step angle. At the smaller average angular velocities the fly produces the responses later than at the higher average angular velocities. The shortest T_{50} of 45 ms was obtained at a movement of average angular velocity w = 600°/s (δt = 5.8 ms and δa = 3.5°). At the higher average angular velocity of w = 1200°/s the 50 % response is reached slightly later: T_{50} = 55 ms. At this pattern speed the response percentage was reduced.

Borst (1986) and Borst and Bahde (1986,1987) measured the dependence of the response moments on the speed of horizontally moving sinewave patterns. The sinewave patterns elicited responses at moments different from those at which the responses to the two stripes were obtained. At stimulation with a high contrast sinewave pattern of spatial wavelength 24° moving at 144°/s the fore legs started their upward movement at 120 ms after the onset of the movement (Borst 1986), whereas, with the stripes moving at average angular velocity w = 150°/s (δt = 5.8 ms, δa = 0.9°) the T_{50} value was 75 ms. Or, at w ≈ 150°/s the responses to the stripe pattern were executed 45 ms earlier than the response to the the sinewave pattern. This 45 ms corresponds with a ≈ 6° smaller angular displacement of the stimulus pattern. At other pattern speeds, similarly, the responses to the moving stripes were executed more rapidly than those to the moving sinewave patterns (see Borst 1986, Borst and Bahde 1987). Apparently inhibitory components play a role in the visual processing of the periodic pattern. The only exception might be the (relatively) rapid responses to the movement of a sinewave pattern of 20° in the results of Borst and Bahde (1987, Fig. 3d).

In the frame by frame method of pattern generation the frequency of the stripe pattern was 173 Hz. With a lower frequency of 44 Hz, obtained by the choice of δt = 23.2 ms and δa = 3.5° and thus resulting in a different 150°/s stepwise movement of the stripes, the responses were

executed after T_{50} = 120 ms. This delayed response moment is similar to the value obtained for the sinewave pattern movement by Borst (1986). Therefore, part of the difference in response times might be due to differences in the methods of pattern generation.

Large steps.

Stripe displacements in horizontal outward direction over angles larger than the interommatidial angle of ≈ 2° did elicit landing responses (Fig. 5,6). Therefore, not only the successive stimulation of photoreceptors with neighbouring visual axes, as was the case at movement in small steps, but also of those with visual axes separated by larger angles contribute to the perception of horizontal movement eliciting the landing response. Apparently, movement detectors with sampling base of 2 times and also 3 times the interommatidial angle are involved in the response.

In the optomotor course control behaviour, similarly, elementary movement detectors with sampling base identical to and larger than the interommatidial angle contribute to the perception of horizontal movement (Buchner 1976). In the turning response, positive interactions occurred at apparent movement of stripes separated by angles up to ca. 8°, whereas at larger angles negative interactions with opposite response direction were obtained (Pick and Buchner 1979).

Measurements on large field movement sensitive H1 neurons in the lobula plate demonstrated that the successive illumination of photoreceptors with visual axes separated by 1, 2 and 3 times the interommatidial angle contribute to the perception of movement (e.g. Lenting 1985).

When the mechanism for movement perception receives not only input from elementary movement detectors with a sampling base identical to the interommatidial angle, but also from elementary movement detectors with sampling base larger than the interommatidial angle, it has the advantage that their parallel contributions will extend the range of movements to which the animal is able to respond.

The amount of movement causing the response.

A movement which causes a response had occurred earlier, on the average 30 ±5 ms earlier. The maximum value of the delay amounts to ≈ 40 ms; no responses occurred later than 40 ms after the termination of the movement stimulus.

Similar delay values have been measured in other visually guided movement dependent behavioural responses. In the turning behaviour during chasing and tracking of *Musca* a delay of 30 ±12.5 ms was found

(Wehrhahn et al. 1982), similar to the values obtained for *Fannia* (Land and Collett 1974). In the optomotor torque response of stationary flying *Musca* a delay of 22.5 - 28 ms has been measured at stimulation by fast jumping stripes, and a delay of 30 - 40 ms at stimulation by continuous moving stripes (Wehrhahn 1981).

The non-specific motor output of the legs in *Calliphora* resulted in a shorter delay with minimum values of 12 - 15 ms at large intensity changes and values of 30 - 40 ms near threshold (Kirschfeld and Vogt 1985), but these responses might depend on a movement independent visual pathway.

A notably longer delay of ≈ 70 ms was found in the forward velocity control of male chasing in *Musca* (Wehrhahn et al. 1982), a result similar to the 60 ms obtained by Wagner (1982) for the deceleration in the pre-landing flight behaviour of *Musca*. In the landing response pathway a delay between stimulus and response of at least 20 ms is suggested by the results of Borst (1986).

The present experiments yield that the small and rather constant number of 3 - 5 successive movement events is sufficient to trigger execution of the landing response when a response delay of only 25 - 35 ms duration is taken into account, irrespective whether neighbouring visual axes or visual axes separated by angles up to 7° are successively stimulated.

References

Beersma DGM, Stavenga DG, Kuiper JW (1975) Organization of visual axes in the compound eye of the fly *Musca domestica* L. and behavioural consequences. J Comp Physiol 102: 305-320

Borst A (1986) Time course of the houseflies' landing response. Biol Cybern 54: 379-383

Borst A, Bahde S (1986) What kind of movement detector is triggering the landing response of the housefly? Biol Cybern 55: 59-69

Borst A, Bahde S (1987) Comparison between the movement detection system underlying the optomotor and the landing response in the housefly. Biol Cybern 56: 217-224

Braitenberg V, Taddei Ferretti C (1966) Landing reaction of *Musca domestica* induced by visual stimuli. Naturwissenschaften 6: 155-156

Buchner E (1976) Elementary movement detectors in an insect visual system. Biol Cybern 24: 85-101

Coggshall JC (1972) The landing response and visual processing in the milkweed bug, *Oncopeltus faciatus*. J Exp Biol 57: 401-413

Eckert H (1980) Orientation sensitivity of the visual movement detection

system activating the landing response of the blowflies, *Calliphora* and *Phaenicia*: a behavioural investigation. Biol Cybern 37: 235-247

Eckert H (1982) Radial pattern expansion drives the landing response of the blowfly, *Calliphora*. Naturwissenschaften 69: 348-349

Eckert H (1983) On the landing response of the blowfly, *Calliphora erythrocephala*. Biol Cybern 47: 119-130

Eckert H, Hamdorf K (1980) Excitatory and inhibitory response components in the landing response of the blowfly, *Calliphora erythrocephala*. J Comp Physiol 138: 253-264

Eckert H, Hamdorf K (1981) The contrast frequency dependence: a criterion for judging the non-participation of neurones in the control of behavioural responses. J Comp Physiol 145: 241-247

Eckert H, Hamdorf K (1983) Does a homogeneous population of elementary movement detectors activate the landing response of blowflies, *Calliphora erythrocephala*? Biol Cybern 48: 11-18

Eckert H, Fligge B, Hamdorf K Excitation and inhibition of the landing response of the blowfly, *Calliphora*. Naturwissenschaften 66: 368-370

Fernandez Perez de Talens A, Taddei Ferretti C (1970) Landing reaction of *Musca domestica*: dependence on dimension and position of the stimulus. J Exp Biol 52: 233-256

Fernandez Perez de Talens A, Taddei Ferretti C (1975) Landing and optomotor responses of the fly *Musca*. In: Horridge GA (ed) The compound eye and vision in insects. pp 490-501 Clarendon Press, Oxford

Fischbach KF (1981) Habituation and sensitization of the landing response of *Drosophila melanogaster*. Naturwissenschaften 68: 332

Franceschini N, Kirschfeld K (1971) Etude optique *in vivo* des éléments photorécepteurs dans l'oeil composé de *Drosophila*. Kybernetik 8: 1-13

Goodman LJ (1960) The landing response of insects. I. The landing response of the fly *Lucilia sericata* and other Calliphorinae. J Exp Biol 37: 845-878

Goodman LJ (1964) The landing response of insects. II. The electrical response of the compound eye of the fly *Lucilia sericata* upon stimulation by moving objects and slow changes in light intensity. J Exp Biol 41: 403-415

Kirschfeld K, Vogt K (1985) The contribution of different colour receptors to a motor output in the fly. J Comp Physiol 157: 417-421

Land MF, Collett TS (1974) Chasing behaviour of houseflies (*Fannia canniculari*s): A description and analysis. J Comp Physiol 89: 331-357

Lenting BPM (1985) Functional characteristics of a wide-field movement processing neuron in the blowfly visual system. Thesis Groningen

Pick B, Buchner E (1979) Visual movement detection under light- and dark-adaptation in the fly, *Musca domestica*. J Comp Physiol 134: 45-54

Taddei Ferretti C, Fernandez Perez de Talens A (1973a) Landing reaction of

Musca domestica, III: dependence on the luminous characteristics of the stimulus. Z Naturforsch 28c: 568-578

Taddei Ferretti C, Fernandez Perez de Talens A (1973b) Landing reaction of *Musca domestica*, IV: A. Monocular and binocular vision. B. Relationship between landing and optomotor reaction. Z Naturforsch 28c: 579-592

Taddei Ferretti C, Fernandez Perez de Talens A (1975) The effect of illumination on the landing response. In: Horridge GA (ed) The compound eye and vision in insects. pp 502-512 Clarendon Press, Oxford

Tinbergen J, Abeln RG (1983) Spectral sensitivity of the landing blowfly. J Comp Physiol 150: 319-328

Wagner H (1982) Flow-field variables trigger landing in flies. Nature 297: 147-148

Wehrhahn C (1981) Fast and slow torque responses in flies and their possible role in visual orientation behaviour. Biol Cybern 40: 213-221

Wehrhahn C, Hausen K, Zanker J (1981) Is the landing response of the housefly (*Musca*) driven by motion of a flow field? Biol Cybern 41: 91-99

Wehrhahn C, Poggio T, Bülthoff H (1982) Tracking and chasing in houseflies (*Musca*). Biol Cybern 45: 123-130

Chapter VII

Spectral Sensitivity of the Landing Blowfly

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Summary. Whether or not colour discrimination is involved in the process of eliciting the landing response of the fly was investigated with rotating linear spiral patterns of different spectral composition. With monochromatic patterns execution of the response depends on the contrast in the pattern: no response occurs within a restricted range of intensity ratios corresponding with low pattern contrast. With dichromatic patterns execution of the response depends on the intensities of the two spectral components (which each dominate half the pattern). No response occurs within a restricted range of intensity ratios; this range characteristically depends on the spectral wavelengths in the pattern. The mean values of these ranges are used to determine a spectral sensitivity distribution of the landing response.

In an alternative procedure the spectral sensitivity was obtained from threshold intensities of a high contrast spiral pattern superimposed on a constant background. The spectra obtained by the two methods are very similar broad-band curves with peaks in the UV and in the blue-green. At the intermediate and low intensity range the contrast thresholds are slightly dependent on the intensity.

Taken together the results suggest that with moving stimuli colour discrimination is absent in the landing response pathway. What is processed are just contrast differences determined by intensity and spectral wavelength.

Introduction

The landing response of flying insects is a behavioural response executed when the animal attempts to perform a landing; a main component of the response is extension of the legs. In tethered, stationary flying animals the landing response can be elicited with moving visual stimuli (Goodman 1960, 1964; Braitenberg and Taddei 1966; Fernandez and Taddei 1970; Coggshall 1972; Taddei and Fernandez 1973a, b; Heisenberg and Buchner 1977; Eckert et al. 1979; Eckert and Hamdorf 1980, 1982; Eckert 1980; Wehrhahn et al. 1981; Fischbach 1981; Wagner 1982). The present study investigates the way in which the spectral information contained in moving visual stimuli is processed in the pathway which serves the landing response of the fly. In the compound eyes of flies at least 5 types of spectral classes of photoreceptor cells are known (e.g. Hardie 1977; Hardie et al. 1979; Smola and Meffert 1979). The spectral information in the signals relayed by the visual cells can in principle be used for colour discrimination tasks as has been shown to occur in social insects. Colour discrimination by flies was studied by Kaiser (1968, 1975) who measured torque responses induced by moving dichromatic patterns. However in this type of optomotor experiments no colour discrimination was observed.

In the experiments described below it was tested whether the landing response can be triggered by colour differences in moving patterns. Necessary for such a test are chromatic patterns with a low subthreshold contrast. Dichromatic patterns, i.e. patterns composed of two intensities of different spectral wavelength, which each dominated half the pattern, and monochromatic patterns, i.e. patterns composed of two intensities of the same spectral wavelength, were used for eliciting the landing response. The reason for also using monochromatic patterns is that dichromatic patterns in general contain both spectral differences and contrast, whereas in monochromatic patterns only contrast is involved. With the latter stimuli

Fig. 1 a, b. Tethered *Calliphora*. Head and thorax are glued to a wire of 0.5 mm diameter. **a** While flying stationarily. Fore and middle legs are flexed and held closely besides and below the body, hind legs are held closely besides the abdomen. **b** While performing the landing response. The legs are extended

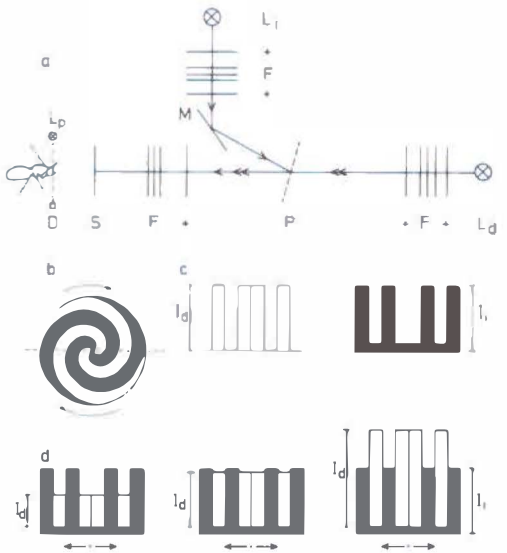

Fig. 2. a Diagram of stimulus equipment. Dichromatic pattern. Slide *P* is imaged on screen *S* twice: by transmission through the transparent parts and by reflection at the silvered parts of the slide. Intensity and spectral wavelength of the two pattern components are set by filters *F*. Optical detector *D* registers foreleg movement. **b** The linear spiral pattern on the screen. White spiral arms correspond with the transparent parts and black spiral arms with the reflecting parts of slide *P*. *Arrows* indicate expanding fashion of rotation around the centre of the pattern. **c** Diagrams of spatial distribution of the intensities along stippled line in **b**. *White:* intensity I_d of spectral wavelength λ_d in the one set of spiral arms and $0.02\,I_d$ of λ_d in the other set ($I_l = 0$). *Black:* intensity I_l of wavelength λ_l in the one set of spiral arms and $0.10\,I_l$ of λ_l in the other set ($I_d = 0$). **d** Diagrams of spatial distribution of the intensity in the dichromatic pattern. *Black* I_l of λ_l (kept constant). *White:* I_d of λ_d. $I_d/I_l = 0.5$, 0.85 and 1.5 respectively. Pattern contrast ($\lambda_l = \lambda_d$) is minimal at $I_d \simeq 0.92\,I_l$ and is high at both low and high I_d. *Arrows* indicate expanding component of movement

the influence of contrast on the landing response is investigated independently of the effect of spectral differences.

If colour discrimination is not involved in the pathway of the landing response, i.e., if only contrast is effective, then the spectral sensitivity characteristics of the landing response can be obtained by determining the pattern intensities at which the response does not occur.

In a separate set of experiments with a pattern consisting of monochromatic pattern components superimposed on a constant background, the spectral sensitivity characteristics and threshold contrast values of the landing response are determined. The results of both types of experiments will be discussed.

Materials and Methods

Animals. Wild type blowflies (*Calliphora erythrocephala*) were used, both males and females. The flies were either caught outdoors or reared in the laboratory; they were kept in cages and lived on liver, sugar, yeast, milkpowder and water.

Preparation and Orientation of the Fly. The experiments were carried out with tethered animals, which performed stationary flight. In order to keep the animals flying stationarily a small piece of aluminum wire was glued to the dorsal side of the head and the anterior part of the thorax (Fig. 1), in most cases under anaesthesia with ether. After a recovery period of at least one day those animals were selected which were flying in a stable manner, i.e. with the fore and middle legs folded and closely held besides the body. No anaesthesia after-effects were observable when compared with flies to which the wire was glued while immobilized by a well-fitting tube.

In the experimental set-up the animal was mounted in a front to back airstream. The airstream produced by a ventilator placed behind the fly, was of help in simulating a normal flight situation. In a number of the experiments a small additional airstream was directed to the antennae from above. The duration of the periods of stable flight were prolonged with this procedure and could last one to several hours.

The visual field of the animal was oriented in a standard manner by examining the deep pseudopupil (Franceschini and Kirschfeld 1971) in an epi-illumination microscope. The animal was adjusted so that the equatorial deep pseudopupil of both eyes became visible in the centre of the microscope. The visual axes of the equatorial ommatidia became directed approximately 20° above the horizontal plane. Subsequently the microscope was removed and in front of the fly a diffusing paper screen was placed.

The visual stimulus pattern was projected on the screen from the other side. A circular area of the mediofrontal visual field of the animal was stimulated so that the centre of the stimulus pattern was displayed at approximately 20° below the equator of the eyes.

Stimulus Patterns. The stimulus patterns were linear spiral patterns rotating in the expanding fashion (Braitenberg and Taddei 1966; Fernandez and Taddei 1970).

In our set-up the spiral pattern on the screen was achieved by projection of a rotating slide. The slide, with spiral arms of equal width, was manufactured by photo-etching the spatial configuration in an aluminum mirror on glass, so that transparent and reflecting spiral arms resulted. The pattern on the screen consisted of two sets of spiral arms corresponding with the transparent and the reflecting parts of the slide respectively. The spatial configuration used in the experiments had 4 spiral arms with a width of 7.5 mm each and hence a spiral pitch of 30 mm. The field diameter was 50 mm. With the screen placed at a distance of 40 mm to the eyes of the fly this configuration resulted in spiral arms of approximately 10° and a pitch of approximately 35°.

Two types of chromatic stimulus patterns were used, each with application of two beams of illumination with different spectral wavelengths. In the first type, the dichromatic patterns, each of the two sets of spiral arms is dominated by a different spectral wavelength. In the second type a high contrast monochromatic spiral pattern is superimposed on a constant background of different spectral composition.

Both types of patterns transform into a purely monochromatic pattern when the spectral wavelengths of both beams of illumination are identical. The intensity of the patterns was from low to intermediate intensity, the sources were not sufficient to cover the high intensity range.

Dichromatic Patterns. The dichromatic patterns are produced by the set-up of Fig. 2 in which the slide P is projected on the screen twice. Light from source L_d (direct beam) is transmitted through the transparent parts of the slide and light from source L_i (indirect beam), incident via mirror M is reflected at the mirroring parts of the slide. The light sources were a 150 W and a 450 W or a 900 W Xe-arc lamp.

The spectral wavelengths of the two beams, λ_d and λ_i respectively, were set by interference filters (Schott, DAL; half-width 8–15 mm). Side bands, if present, were suppressed by broad-band absorption filters. The intensities, I_d and I_i respectively, were set by density filters in calibrated steps of approximately 10%.

The high contrast images of the two pattern components were fused into one single pattern as accurately as possible. Small image imperfections at the boundaries between adjacent spiral arms were unavoidable. They introduced inaccuracies which seemed to be acceptable for the type of experiment.

Monochromatic Spiral Superimposed on Background. The same light sources, lenses, interferenee and density filters as used in the set-up of Fig. 2 were employed in the set-up of Fig. 3. However, the first light source now creates a high contrast monochromatic spiral pattern of spectral wavelength λ_s and

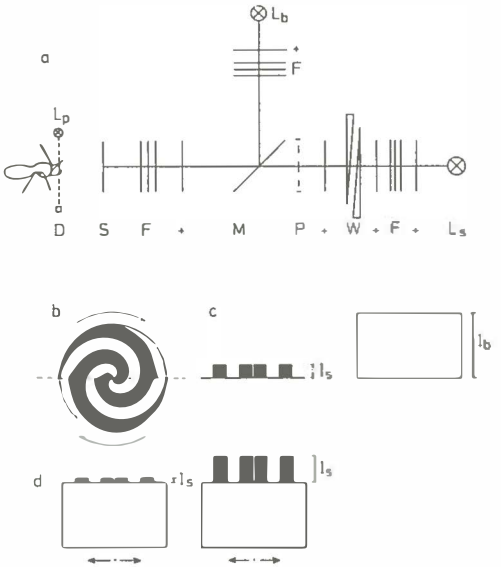

Fig. 3. **a** Diagram of stimulus equipment. Spiral superimposed on background. Slide P is imaged on screen S via source L_s. L_b provides the background. Intensity and spectral wavelength of the two pattern components are set by wedge W and filters F. Optical detector D registers foreleg movement. **b** The linear spiral pattern on the screen. *White spiralarms:* background; *black arms* spiral + background. **c** Diagrams of spatial distribution of the intensities along stippled line in **b**. *White:* I_b of wavelength λ_b in both sets of spiral arms ($I_s = 0$). *Black:* I_s of wavelength λ_s in the one set of spiral arms and 0.02 I_s in the other set ($I_b = 0$). **d** Diagram of spatial distribution of the intensities in the spiral superimposed on background. *White:* I_b of λ_b (kept constant). *Black:* I_s of λ_s. Pattern contrast increases with I_s. *Arrows* indicate expanding component of movement

intensity I_s which is superimposed at a background of wavelength λ_b and intensity I_b, created by the second light source, now called L_b. The latter light beam is projected on the screen via half-mirror M. The intensity I_s is set by a grey wedge W in calibrated steps of 1%. The spiral component and background component fused into one pattern having nearly negligible imperfections, which is an advantage of this type of pattern, when compared with the dichromatic type of pattern.

Pattern Contrast. The contrast in a pattern having two intensities is defined by

$$c = |(I_1 - I_2)/(I_1 + I_2)|. \quad (1)$$

When the intensities I_1 and I_2 are of different spectral wavelength and α is the effectivity of the second beam as compared to the first beam the visually effective contrast becomes

$$c = |(I_1 - \alpha I_2)/(I_1 + \alpha I_2)|. \quad (2)$$

In the dichromatic patterns (set-up of Fig. 2) I_1 and I_2 were not purely monochromatic. Small background contributions were present arising from reflection at the surfaces of the glass slide and from scattered light.

It turned out that $I_1 = I_i + 0.02\ I_d$ and $I_2 = I_d + 0.10\ I_i$. Pattern contrast was calculated with

$$c = |(0.90\, I_i - 0.98\, I_d)/(1.10\, I_i + 1.02\, I_d)| \quad (3)$$

and the effective contrast with

$$c = |(0.90\, I_i - 0.98\, \alpha I_d)/(1.10\, I_i + 1.02\, \alpha I_d)|. \quad (4)$$

This formula is used for the data presented in Figs. 4 and 6. However, image imperfections at the boundaries of the spiral arms and small local intensity differences did slightly contaminate the pattern. Consequently zero contrast, expected from the contrast formula when $I_d = 0.92\, I_i$, could not be realized in practice. The dichromatic pattern had a contrast minimum at approximately $I_d \sim 0.92\, I_i$. Experiments with monochromatic spirals on a background suggested that the image imperfections in the dichromatic patterns were as effective as a few percent contrast. In the contrast formula this inaccuracy was not taken into account. Yet, near minimum contrast the contrast in the pattern turned out to be subthreshold for the landing response system, so that the spectral properties could be investigated successfully.

With the monochromatic pattern on a background (set-up of Fig. 3) the intensities of the two pattern fields become $I_1 = I_b + 0.02\, I_s$ and $I_2 = I_b + I_s$. Thus the contrast described with Eq. (1) becomes

$$c = 0.98\, I_s/(2\, I_b + 1.02\, I_s) \simeq I_s/(I_s + 2\, I_b) \quad (5)$$

This formula is used in the calculations of the contrast values presented in Fig. 11.

Experimental Procedure. In the dichromatic pattern experiments it was tested whether the stimuli presented are sufficient or not sufficient to elicit the landing response.

In each test stimulus parameters intensity and spectral wavelength of both beams (I_d, λ_d, I_i and λ_i) were set while the pattern rotation speed was subthreshold for the landing response (0.25 rps). After an adaptation period of 15–60 s the animal was tested. During the test rotation speed of the pattern gradually increased to 10 rps in 5 s. It was registered whether or not the stimulus was sufficient to elicit the landing response. Subsequent to each test rotation speed was reset to 0.25 rps. Series of tests were performed in which I_i, λ_i and λ_d were kept constant and I_d was varied in steps of approximately 10%. Hence pattern contrast changed with I_d and was high at low values of I_d, low at intermediate I_d and high again at high I_d.

In the experiments with monochromatic spirals superimposed on a background the intensity of the spiral at which the landing response is executed was measured. Intensity and spectral wavelength of the background and spectral wavelength of the spiral (I_b, λ_b and λ_s) were set while the pattern rotated at constant velocity of 5 rps (above threshold). The intensity of the spiral I_s was set at a very low sub-threshold value and kept constant during an adaptation period of 15–60 s. Subsequently the intensity of the spiral was increased in 1% steps (5 steps/s) till the landing response was elicited. When the landing response occurred the intensity of the spiral was reset to the low subthreshold value.

Detection of the Response. A detector unit, consisting of a narrow beam of light projected on an optical detector, was placed just beside the head of the fly. The detector was activated when the beam of light was interrupted due to foreleg movement of the animal.

The beam was oriented vertically, such that interruption of the beam at extension of the legs occurred at the halfway position of the horizontal component of the foreleg movement. Responses did occur in normal flies with uncovered compound eyes and ocelli. No responses were obtained when both compound eyes were painted black. When the ocelli were painted black, the responses were identical to those of animals with uncovered ocelli, but these animals showed a decreased flight stability.

Hence, it was concluded that the compound eyes are the essential visual component in the landing response pathway, whereas contribution of the ocelli is absent or at least subthreshold.

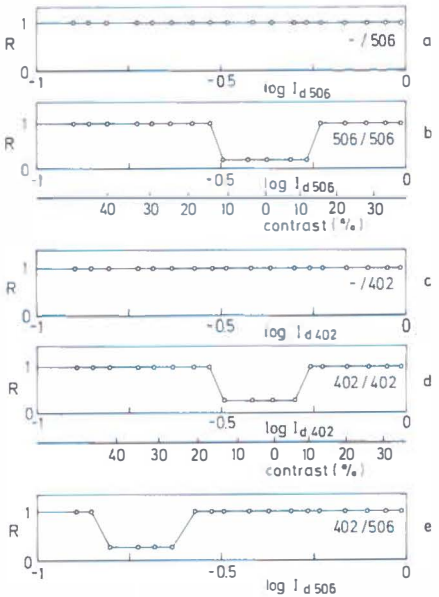

Fig. 4a–e. Landing response 1 = response elicited, 0 = absence of response. **a** High contrast monochromatic 506 nm pattern ($I_i = 0$). Response at all I_d. **b** Variable contrast monochromatic 506 nm pattern ($\lambda_i = \lambda_d$ and $I_i \simeq 0.5\, I_d$max). The response is absent in the low contrast range. *Lower scale:* contrast, accurate to a few percent. **c** As **a** but $\lambda_d = 402$ nm. **d** As **b** but $\lambda_d = \lambda_i = 402$ nm. **e** Dichromatic pattern ($\lambda_d = 506$ nm, $\lambda_i = 402$ nm and $I_i \simeq 0.5\, I_d$max). Absence of response in restricted range of I_d

Results

Is the Landing Response Colour-Dependent?

Experiments with both monochromatic and dichromatic patterns are performed in order to investigate whether spectral wavelength differences are perceived as different. Results of monochromatic pattern experiments ($\lambda_i = \lambda_d$) demonstrating contrast dependency of the landing response are presented first. Results of dichromatic pattern experiments ($\lambda_i \neq \lambda_d$) are decribed next.

Monochromatic Stimuli

It was investigated for a high contrast monochromatic spiral whether or not the landing response

occurred at different pattern intensities. The indirect beam of the set-up of Fig. 2 was switched off ($I_i = 0$) and the direct beam was kept at different intensities I_d of $\lambda_d = 506$ nm. In each test the rotation speed of the pattern increased in 5 s from a value of low subthreshold (0.25 rps) to a value above threshold (10 rev/s). In the intervals between successive tests the intensity I_d was changed in steps of approximately 10%.

The results (Fig. 4a) show that the high contrast 506 nm pattern was sufficient to elicit the landing response at all intensities tested.

Subsequently experiments were performed in which pattern contrast was varied. The intensity of the indirect beam was kept constant ($I_i \simeq \frac{1}{2} I_{d\,max}$). The contrast in the pattern was changed by changing I_d in steps of approximately 10%. An example of the results is given in Fig. 4b. Landing responses occurred for both high and low values of I_d, but not for values of I_d close to the values of I_i. The contrast values calculated with Eq. (3) are indicated in the figure and suggest that at least approximately 15% contrast was necessary to elicit the landing response. Figure 4c and d show the results for a pattern wavelength of 402 nm. Again, for the high contrast spiral pattern responses were obtained at all intensities tested (Fig. 4c) and none occurred for low contrast values (Fig. 4d).

Monochromatic experiments performed with other spectral wavelengths yielded similar results, high contrast was sufficient to elicit the landing response and low contrast was not sufficient to elicit the landing response. Contrast dependency of the response suggests that the pattern configuration used is sufficient to test the influence of spectral differences.

Dichromatic Stimuli

The dichromatic stimuli were obtained by combining two different spectral wavelengths in the pattern. In the series of tests presented first the spectral wavelengths 506 nm and 402 nm were selected, the same wavelengths as used in the experiments of Fig. 4a–d. A constant intensity I_i (402) in the indirect beam was combined with different intensities I_d (506) in the direct beam ($I_i(402) \simeq \frac{1}{2} I_d(506)_{max}$). Figure 4e shows the results. Landing responses were registered at low $I_d(506)$ and at high $I_d(506)$ but in a range of intermediate $I_d(506)$ no landing response could be elicited.

If the two wavelengths 402 nm and 506 nm would have been evaluated as different colours, then we would have expected also landing responses at intermediate I_d values. Hence the experimental results of Fig. 4 suggest that for 402 nm and 506 nm discrimination between colours is not involved in eliciting the landing response. The values of $I_d(506)$ at which no response is registered in the 506/506 nm contrast experiment (Fig. 4b) are approximately 3 times higher than those in the dichromatic 402/506 nm experiment (Fig. 4e). This suggests that 506 is approximately 3 times more effective to the animal than 402 nm (see further Sect. 2). Underlying this conclusion is the hypothesis that the monochromatic combinations 402/402 nm and 506/506 nm and the dichromatic

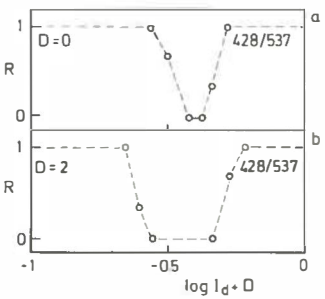

Fig. 5. Dichromatic pattern ($\lambda_d = 537$ nm and $\lambda_i = 428$ nm) at two intensity levels ($D = 0$ and $D = 2$ respectively). Absence of landing response in restricted range of I_d. The no-response range is wider at the lower intensity level

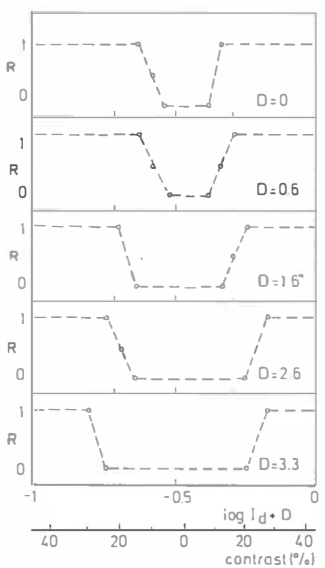

Fig. 6. Landing response dependence on intensity level of monochromatic pattern ($\lambda_d = \lambda_i = 506$ nm). At lower intensity levels more contrast is necessary to elicit the response

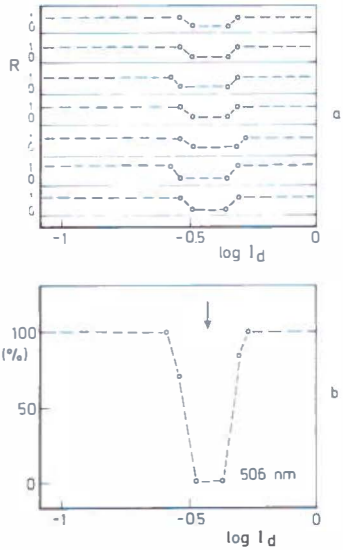

Fig. 7a, b. Determination of the mean intensity $\bar{I}_m$ of the I_d range where no landing response occurs. **a** Results of 7 test runs, averaged in **b**. *Arrow* indicates $\bar{I}_m$, further details see text

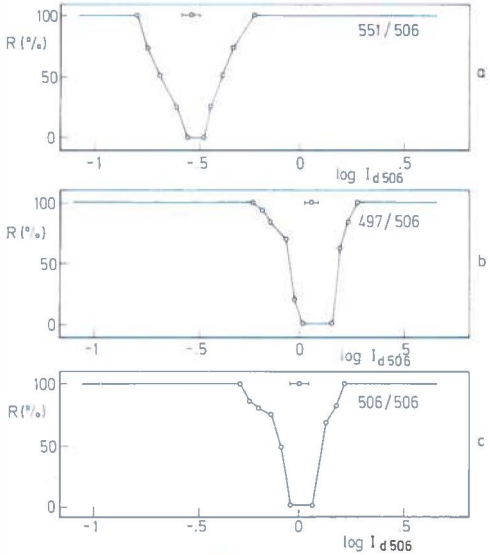

Fig. 8a–c. Mean intensity $\bar{I}_m$ of no-response range: open circle above the through, horizontal bar: twice the standard deviation. $\lambda_d = 506$ nm. **a** $\lambda_i = 551$ nm, 8 runs from 4 animals. **b** $\lambda_i = 497$ nm, 17 runs from 6 animals. **c** $\lambda_i = 506$ nm, 31 runs from 8 animals

combination 402/506 nm are processed in essentially identical ways.

The results of experiments with wavelengths 428 and 537 nm are presented in Fig. 5. The experiments were performed at 2 intensity levels ($D=0$ and $D=2$ respectively) in order to examine whether the choice of the intensity level was crucial for the results. It appeared that again ranges of intensity ratios are found at which no landing response can be elicited. It seemed, however, that increased intensity narrows the no-response range.

Further dichromatic pattern experiments revealed that, for spectral wavelengths between 350 and 600 nm, each of the wavelength combinations tested resulted in a range of intensity ratios at which no landing response is measured. This was also the case for combinations in which the one component was monochromatic and the other component was white light.

Determination of No-Response Intensities

Series of experiments were performed in order to determine the mean values of the ranges of intensities in the pattern at which no landing response is executed. With an interpolation procedure these mean values were obtained from the animal's responses. The stimuli having intermediate value of I_i, were presented in a decreasing contrast test sequence: with high value of I_d each test was followed by a test with approximately 10% lower I_d and on the other hand with low I_d each test was followed by a test with an approximately 10% higher I_d. In this procedure two threshold values were obtained, the highest value of low I_d and the lowest value of high I_d at which a landing response was executed, called I_l and I_u respectively. In the high intensity range no landing response occurred below I_u and in the low intensity range no landing response occurred above I_l. The mean value of the no-response intensity range I_m, of each series of tests was obtained by

$$I_m = \sqrt{I_l \times I_u}. \tag{6}$$

The I_m values of different series of tests were averaged. Dichromatic stimuli were applied to determine the relative sensitivity for each spectral wavelength.

Monochromatic Stimuli

The animals were tested first with monochromatic patterns ($\lambda_i = \lambda_d$). The intensity of the indirect beam I_i was fixed, while the intensity I_d of the direct beam was changed and hence the contrast in the pattern. Results of series of tests performed at in-

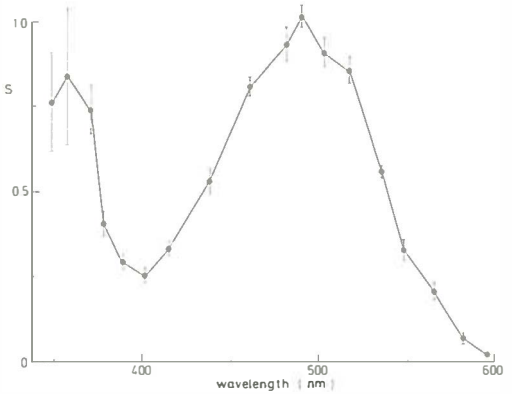

Fig. 9. Spectral sensitivity of landing response system, dichromatic patterns, *Calliphora*. Data based on 8 animals. Sensitivity values calculated from $\bar{I}_m$ values of at least 8 sets of threshold values from 4 animals, $\lambda_d = 506$ nm

tensity levels over a range of 3.3 log units are presented in Fig. 6. The figure illustrates that the no-response range widens when the intensity is dropped, a result similar to that of Fig. 5.

More contrast seems to be necessary to elicit the landing response at lower intensity levels. Hence, the threshold values I_u and I_l depend slightly on intensity. Threshold values obtained in 7 series of tests with a 506/506 nm pattern are presented in Fig. 7. The I_m values of the no-response ranges were interpolated and the average value $\bar{I}_m$ was calculated. $\bar{I}_m$ is indicated by an arrow in Fig. 7.

All 506/506 nm monochromatic tests together yielded $\bar{I}_m = 0.90\ I_i \pm 0.03\ I_i$. In 402/402 nm monochromatic tests $\bar{I}_m = 0.89\ I_i \pm 0.05\ I_i$ was obtained. These values are slightly lower but close to $I_d = 0.92\ I_i$, the value which indicates the contrast minimum of the pattern, as predicted from the contrast formula. The results suggest that $\bar{I}_m$ is nearby identical to the contrast minimum of the pattern.

Dichromatic Stimuli

Dichromatic experiments are performed with patterns of which one spectral wavelength, λ_i, was changed and the other spectral wavelength, λ_d, kept constant. In each series of tests the intensity I_i was constant and the intensity I_d changed stepwise. A range of I_d values corresponding with no-response was obtained in all series of tests, as was already mentioned. The threshold values I_u and I_l were used to calculate the relative sensitivity for each wavelength λ_i. Results of 3 spectral wavelength combinations are presented in Fig. 8. Figure 8c, where $\lambda_i = \lambda_d = 506$ nm represents not a dichromatic case but is the reference for the experiments where $\lambda_i \neq 506$ nm. The $\bar{I}_m$ value in Fig. 8c corresponds with $\log I_d = 0$; Fig. 8a and 8b are scaled accordingly. It thus appears that the no-response range for $\lambda_i = 551$ nm (Fig. 8a) is shifted to much lower value and for $\lambda_i = 496$ nm (Fig. 8b) to slightly higher values of $\log I_d$. More I_d (506 nm) has to be applied because of the higher sensitivity for 497 nm and less I_d (506) because of the lower sensitivity for 551 nm.

The spectral sensitivity was calculated in a similar way for a number of wavelengths over the range between 350 nm and 600 nm. In practice the intensity I_i was not fully identical for all wavelengths. The intensities $I_i(\lambda)$ were selected such that the corresponding $\bar{I}_m$ values did vary less than a factor 4 throughout the whole wavelength range. The relative spectral sensitivity distribution normalized at wavelength 497 nm is presented in Fig. 9. A double-peaked curve resulted, with a broad-band peak in the blue-green and an about equally high but more variable peak in the ultraviolet. The peaks are separated from each other by a distinct trough at about 400 nm.

Stimulation with Rotating Monochromatic Pattern on Constant Background

Spectral Sensitivity

Stimuli consisting of a background to which a monochromatic spiral component is added are used. They have the advantage that the accuracy of the superposition of the two pattern components is better than in the dichromatic stimuli, that the movement of the pattern can be kept constant, and that the spectral composition in the pattern is less variable because of the constant background. With these patterns colour discrimination cannot be tested, however.

A spectral sensitivity distribution of the landing response has been determined using as the background a constant intensity of longer wavelengths (570–710 nm).

The intensity of the spiral component I_s was increased from below threshold, until at threshold I_t the animal executed the landing response. The threshold values I_t were used to calculate the sensitivity at spectral wavelength λ_s. A procedural difficulty in this approach concerned the choice of the subthreshold starting value of I_s. With too high values considerable drifting and high threshold values were obtained in pilot experiments. In the following detailed experiments the subthreshold in-

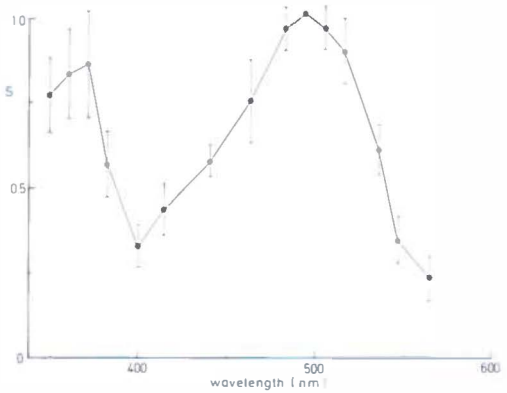

Fig. 10. Spectral sensitivity of landing response system, monochromatic spirals on constant background of broadband wavelengths λ_b = 570–720 nm. *Calliphora*. Each data point based on 45 threshold values (9 animals)

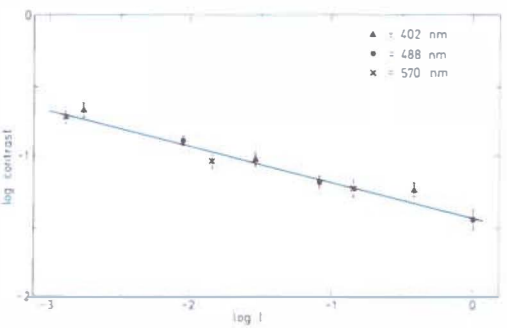

Fig. 11. Intensity dependence of landing response, threshold contrast for monochromatic spiral on background $\lambda_s = \lambda_b$ at 402 nm, 488 nm and 570 nm respectivily. Each data point based on 15 threshold values (3 animals)

tensities were chosen at least a factor of 8 below threshold.

The relative sensitivity at each wavelength was obtained with

$$S(\lambda) = \bar{I}_t(497)/I_t(\lambda). \quad (7)$$

The resulting spectral sensitivity curve is presented in Fig. 10. The curve with two peaks, one in the ultraviolet and one in the blue-green region of the spectrum is similar to that of Fig. 9.

Intensity Dependence of the Threshold Contrast

Patterns with $\lambda_b = \lambda_s$ were applied to determine monochromatic threshold contrast values at which the landing response is executed.

The intensity of the background I_d was kept constant and the intensity of the spiral raised in 1% steps from low subthreshold until threshold. The experiments were performed at different intensity levels by inserting density filters which attenuated both I_b and I_s.

The threshold contrasts as a function of intensity were determined at wavelengths 402, 488, and 570 nm respectively. The animal was adapted to every new wavelength at the highest intensity available during 5 min and to each background intensity during 2 min in order to erase possible aftereffects of previous tests.

The threshold contrast values, calculated with Eq. (5) are presented in Fig. 11. It appears that threshold contrast decreases with increasing intensity. The intensity dependence is not noticeably different for the various spectral wavelengths. Contrast at threshold decreases only $\simeq 0.7$ log unit over a 3 log units intensity range.

Discussion

A main conclusion drawn from the results of the experiments with dichromatic moving patterns is that spectral differences are not evaluated as colour differences in the visual pathway leading to the landing response. This conclusion is based on the absence of the landing response in a specific range of intensity ratios of the dichromatic pattern; this holds for all the dichromatic patterns used. Since with monochromatic patterns absence of the landing response was measured in the range of intensity ratios where pattern contrast was low, the results of the dichromatic experiments are interpreted likewise, namely that absence of responses occurred at low pattern contrast. An indication for colour discrimination would have been obtained when landing responses occurred at all intensity ratios of the dichromatic pattern implying the independence of the landing response from contrast in the pattern.

With the dichromatic patterns a spectral sensitivity curve was determined from the no-response ranges using the interpolation method; the mean of the intensity ratios at the lower and upper thresholds was calculated. The mean corresponds to approximately minimal contrast as follows from the monochromatic experiments. The sensitivity spectrum thus obtained (Fig. 9) compares very well with the spectrum deduced from experiments with a spiral pattern superimposed on a background (Fig. 10). The good correspondence of the two spectra reinforces the above conclusion, namely that colour discrimination is absent and that only

sufficient contrast in the pattern elicits the landing response.

The spectral sensitivity of the landing response can be compared with spectral sensitivities measured from single visual sense cells by intracellular electrophysiology. The peripheral sense cells R1–6 belong to a type generally described as having a UV + blue-green peaked sensitivity (e.g. Burkhardt 1962; Dörrscheidt-Käfer 1972; Horridge and Mimura 1975; Hardie 1977, 1979; Smola and Meffert 1979). The central sense cells R7 and R8 belong to at least four different spectral types, having sensitivity peaks in the UV, the UV + blue, the blue and in the green (Meffert and Smola 1976; Hardie 1977, 1979; Hardie et al. 1979; Smola and Meffert 1979). The absolute sensitivities of R7's and R8's seem to be not higher than that of R1–6 cells.

Since the sensitivity spectra of the landing response and that of the receptors R1–6 are very similar it is tempting to hypothesize that only receptor type R1–6 contributes to the visual pathway leading to the landing response. A simple summation of receptor outputs of R1–6, having the same visual axis, seems to be physiologically realistic because of their convergence onto the first optic ganglion. However, an additional contribution of the central receptors R7 and/or R8 cannot be rejected on the basis of the spectral sensitivity distribution, and even involvement of exclusively receptors R7 and/or R8 remains an alternative possibility. For, if all receptors, R1–8 together, contribute to the landing response pathway the spectral sensitivity distribution is expected to be UV + blue-green, double-peaked because of the dominance in number of R1–6. Alternatively and dependent on their absolute sensitivities and their relative numbers, the spectral sensitivity distribution of just R7's and R8's together might yield a UV + blue-green double-peaked curve also, similar to the one obtained for the landing response.

In *Drosophila* pre-adaptation with blue light resulted in a decreased sensitivity of the landing response system interpreted as being due to a dominant contribution of receptors R1–6 in the landing response pathway (Heisenberg and Buchner 1977). Because of the similarities between the visual systems of *Drosophila* and *Calliphora* we would suggest that at least R1–6 receptors contribute in the landing response pathway of *Calliphora*.

The results of Fig. 11 illustrate the intensity dependence of threshold contrast. At higher intensity less contrast is needed to obtain responses, i.e. sensitivity increases with intensity. The dependence of $\log \Delta I$ on background $\log I$ yields a slope of approximately -0.75. Dörrscheidt-Käfer (1972) calculated a similar slope of -0.67 for contrast changes in receptor potential of visual sense cells R1–6 (*Calliphora*, mutant chalky). Incidently, from Dörrscheid-Käfer's Fig. 10 a change in receptor potential of approximately 1 mV is calculated, corresponding to the threshold contrast for eliciting the landing response. Furthermore, the contrast sensitivity as derived for the landing response is consistent with the contrast sensitivity of the lamina monopolar cells reported by Laughlin and Hardie (1978). In conclusion, the intensity dependence of threshold contrast as expressed in the landing response is not conflicting with the hypothesis that receptors R1–6 and lamina monopolar cells are involved in the landing response pathway.

Kaiser (1968, 1975) performed optomotor experiments using methods similar to those applied in the landing response experiments presented in this report. With monochromatic and dichromatic moving stripe patterns the torque response was determined in the blowfly *Phormia*. At various spectral wavelength combinations the intensity ratios were determined where the elicited torque was minimal. Kaiser (1968, 1975) concluded from the obtained results that colour perception is not (or hardly at all) involved in the torque response when induced by moving stimuli. This conclusion is in line with the above for the *Calliphora* landing response. Kaiser determined the minima of time averaged torque, but never obtained a zero torque. The spectral sensitivity curve calculated from the torque minima was a double-peaked curve, in the UV and in the green, similar to the spectrum obtained at threshold using monochromatic patterns with high contrast. Both curves show a UV peak distinctly lower than the green peak. This may be a characteristic of *Phormia*, but an alternative explanation may be found in a low vitamin A diet (Steiner 1942; Razmjoo and Hamdorf 1976; Stark et al. 1977).

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References

Braitenberg VB, Taddei Ferretti C (1966) Landing reaction of *Musca domestica*. Naturwissenschaften 53:155

Burkhardt D (1962) Spectral sensitivity and other response characteristics of single visual cells in the arthropod eye. Symp Soc Exp Biol 16:86–109

Coggshall JC (1972) The landing response and visual processing in the milkweed bug *Oncopeltus fasciatus*. J Exp Biol 57:401–413

Daumer K (1956) Reizmetrische Untersuchungen des Farbensehens der Bienen. Z Vergl Physiol 38:413–478

Dörrscheidt-Käfer M (1972) Die Empfindlichkeit einzelner Photorezeptoren im Komplexauge von *Calliphora erythrocephala*. J Comp Physiol 81:309–340

Eckert H (1980) Orientation sensitivity of the visual movement detection system activating the landing response of the blowflies, *Calliphora* and *Phaenicia*: A behavioural investigation. Biol Cybern 37:235–247

Eckert H, Hamdorf K (1980) Excitatory and inhibitory response components in the landing response of the blowfly *Calliphora erythrocephala*. J Comp Physiol 138:253–264

Eckert H, Hamdorf K (1982) Contrast frequency dependence. A criterion for judging the non-participation of neurons in the control of behavioural responses. J Comp Physiol 145:241–247

Eckert H, Flügge B, Hamdorf K (1979) Excitation and inhibition in the activation of the landing response of the blowfly, *Calliphora*. Naturwissenschaften 66:368–370

Fernandez Perez de Talens A, Taddei Feretti C (1970) Landing reaction of *Musca domestica*: dependence on dimension and position of the stimulus. J Exp Biol 52:233–256

Fischbach KF (1981) Habituation and sensitization of the landing response of *Drosophila melanogaster*. Naturwissenschaften 68:332

Franceschini N, Kirschfeld K (1971) Les phénomènes de pseudopupille dans l'œil composé de *Drosophila*. Kybernetik 9:159–182

Goodman LJ (1960) The landing responses of insects. I. The landing response of the fly *Lucilia sericata* and other Calliphorinae. J Exp Biol 37:854–878

Goodman LJ (1964) The landing responses of insects. II. The electrical response of the compound eye of the fly *Lucilia sericata* upon stimulation by moving objects and slow changes of light intensity. J Exp Biol 41:403–415

Hardie RC (1977) Electrophysiological properties of R7 and R8 in dipteran retina. Z Naturforsch 32c:887–889

Hardie RC (1979) Electrophysiological analysis of fly retina. I. Comparative properties of R1–6 and R7 and R8. J Comp Physiol 129:19–33

Hardie RC, Franceschini N, McIntyre PD (1979) Electrophysiological analysis of fly retina. II. Spectral and polarization sensitivity in R7 and R8. J Comp Physiol 133:23–39

Heisenberg M, Buchner E (1977) The role of retinula cell types in visual behaviour of *Drosophila melanogaster*. J Comp Physiol 117:127–162

Helversen O von (1972) Zur spectralen Unterschiedsempfindlichkeit der Honigbiene. J Comp Physiol 80:439–472

Horridge GA, Mimura K (1975) Fly photoreceptors. I. Physical separation of two visual pigments in *Calliphora* retinula cells 1–6. Proc R Soc Lond [Biol] 190:211–224

Kaiser W (1968) Zur Frage des Unterscheidungsvermögen für Spektralfarben: Eine Untersuchung der Optomotorik der königlichen Glanzfliege *Phormia regina* Meig. Z Vergl Physiol 61:71–102

Kaiser W (1975) The relationship between visual movement detection and colour vision in insects. In: Horridge GA (ed) The compound eye and vision of insects. Clarendon Press, Oxford, pp 359–377

Laughlin SB, Hardie RC (1978) Common strategies for light adaptation in the peripheral visual system of fly and dragonfly. J Comp Physiol 128:319–340

McCann GD, Arnett DW (1972) Spectral and polarisation sensitivity of the dipteran visual system. J Gen Physiol 59:534–558

Meffert P, Smola U (1976) Electrophysiological measurements of spectral sensitivity of central visual cells in the eye of the blowfly. Nature 260:342–344

Razmjoo S, Hamdorf K (1976) Visual sensitivity and variation of total photopigment content in the blowfly photoreceptor membrane. J Comp Physiol 105:279–286

Smola U, Meffert P (1979) The spectral sensitivity of the visual cells R7 and R8 in the eye of the blowfly *Calliphora erythrocephala*. J Comp Physiol 133:41–52

Stark WS, Ivanyshyn AM, Greenberg RM (1977) Sensitivity and photopigments of R1–6, a two-peaked photoreceptor in *Drosophila*, *Calliphora* and *Musca*. J Comp Physiol 121:289–305

Steiner G (1942) Eine Zuchtweise für Fleischfliegen. Zool Anz 138:97–106

Taddei-Feretti C, Fernandez Perez de Talens A (1973) Landing reactions of *Musca domestica*. III. Dependence on the luminous characteristics of the stimulus. Z Naturforsch 28c:568–578

Taddei-Feretti C, Fernandez Perez de Talens A (1973) Landing reactions of *Musca domestica*. IV. A. Monocular and binocular vision. B. Relationship between landing and optomotor reaction. Z Naturforsch 28c:579–592

Wagner H (1982) Flow-field variables trigger landing in flies. Nature 297:147–148

Wehrhahn C, Hausen K, Zanker J (1981) The landing response of the housefly (*Musca*) as driven by motion of a flow field. Biol Cybern 41:91–99

Samenvatting.

Dit proefschrift behandelt een tweeledig onderzoek dat verricht is aan respectievelijk de visuele zintuigcellen in het samengestelde oog en de landingsreactie van vliegen.

Het eerste deel, hoofdstuk 2-4, betreft optische metingen aan de energievoorziening van de visuele zintuigcellen in het oog van de bromvlieg. Visuele zintuigcellen bevatten een grote hoeveelheid van het lichtabsorberende, visuele pigment. Absorptie van licht door het visuele pigment wekt in de zintuigcel een electrisch signaal op, dat vervolgens doorgegeven wordt aan het visuele deel van de hersenen. Voor deze lichtgevoeligheid van de visuele zintuigcel is energie nodig. De energievoorziening wordt verzorgd door de mitochondria, kleine gespecialiseerde onderdeeltjes van de cel. Daarin wordt energie geproduceerd in een voor de cel hanteerbare vorm, energierijk fosfaat. Dit als product van oxidatieve metabole processen, waarbij ondermeer zuurstof wordt gebruikt en koolzuur afgegeven, en wel via de tracheeën, die tot in het oog doorlopen. Bij de productie van energierijk fosfaat zijn flavoproteïnes betrokken. De activiteit van deze enzymen kan gemeten worden met behulp van optische methodes, met name door fluorescentie metingen. Uit metingen van de fluorescentie van het oog van intacte vliegen is bij dit onderzoek gebleken dat de flavoproteïnefluorescentie, en dus de energieproductie, afhangt van de belichtingstoestand van de zintuigcellen. De gevoeligheid van de mitochondriële activiteit voor belichting met verschillende zuivere kleuren blijkt hoog te zijn voor ultraviolet en groen licht, lager voor blauw licht en veel lager voor rood licht. Deze spectrale gevoeligheid komt overeen met die van het visuele pigment te samen met die van het bijbehorende ultratravioletgevoelige antenne-pigment van de zintuigcel. Dit geldt zowel bij plotselinge belichting vanuit donker als voor een toestand waarbij de zintuigcel door zgn. adaptatie zijn gevoeligheid geleidelijk heeft aangepast aan de belichting. Bij afwezigheid van zuurstof worden de mitochondriële processen geheel stilgelegd. Dit blijkt zowel uit de fluorescentie metingen aan de flavoproteïnes als uit absorptiemetingen aan de zgn. cytochromen, stoffen die eveneens direct bij de energieproductie betrokken zijn. Waarschijnlijk neemt bij belichting van de zintuigcel de concentratie van calcium-ionen in de cel toe. Daardoor stijgt de mitochondriele activiteit en dus de productie van metabole energie. Regulatie van cellulaire processen door calcium is een biologisch algemeen gebruikte manier.

In het tweede deel, hoofdstuk 5-7, worden experimenten beschreven die verricht zijn aan het landingsgedrag van de bromvlieg en de huisvlieg. Ze zijn uitgevoerd met stationair vliegende vliegen, die door een zowel aan de rug als aan de kop vastgeplakt draadje met de proefopstelling verbonden

waren. Tijdens de vlucht zijn bij de vlieg de voorste en middelste pootparen opgevouwen naast het lichaam, de achterpoten steken naar achteren, naast en achter het achterlijf. Het uitvoeren van de landingsreactie gaat gepaard met het plotseling uitsteken van de poten en een remmende verandering van de vleugelslag. Daarmee tracht het dier veilig te landen, of een niet meer te vermijden botsing met een zo gering mogelijke snelheid te laten plaatsvinden. De landingsreacties zijn gemeten met een speciale optische pootbewegingsdetector. De reactietijd van de vlieg is kort. Het kost ongeveer 35 ms om een begin te maken met het uitsteken van de poten, welke beweging na nog eens 45 ms voltooid is. Deze gedragsreactie kan met visuele stimuli opgewekt worden, en dus kan de landingsreactie inzicht verschaffen in de werking van het visuele systeem. Het blijkt dat bewegingen, en plotselinge afname van de lichtintensiteit, in staat zijn de landingsreactie op te wekken. Bewegende patronen hoeven hiervoor niet een naderende beweging uit te voeren: uitwaartse bewegingen waarbij de afstand tot de vlieg niet verandert zijn eveneens effectieve stimuli. Deze laatste kunnen nadering suggereren. Inwaartse bewegingen zijn niet slechts onvoldoende om de landingsreactie op te wekken, maar hebben juist een remmende invloed. Voor in horizontale richting, uitwaarts bewegende strepen is de afhankelijkheid van de bewegingssnelheid geanalyseerd. Bij stapvormige bewegingen met gemiddelde hoeksnelheden boven ca. 40 °/s is steeds vrijwel dezelfde hoeveelheid beweging nodig om de landingsreactie op te wekken, ongeacht de grootte van de stapjes en de tijdsduur tussen de stapjes waaruit de beweging is opgebouwd. Op grond hiervan kan geconcludeerd worden dat bij het zien van beweging zowel zintuigcellen met naburige blikrichtingen betrokken zijn als zintuigcellen met verder uiteenliggende blikrichtingen. Samen stellen ze de vlieg in staat zelfs bij zeer snelle bewegingen te reageren. Landingsreacties zijn ook opgewekt met als bewegend patroon een ronddraaiende spiraal waarvan de naburige spiraalarmen verschillende zuivere kleuren hebben. Met deze twee-kleurige patronen is onderzocht of vliegen bij het uitvoeren van een landingsreactie het verschil in kleur benutten. Dit blijkt niet het geval. Slechts het helderheidscontrast in de patronen blijkt de reacties te bepalen. Meting van dit contrast levert de spectrale gevoeligheid van de landingsreactie op. Deze toont een hoge gevoeligheid voor ultraviolet en groen licht, een lagere gevoeligheid voor blauw licht en een veel lagere voor rood licht. Dit demonstreert dat de spectrale gevoeligheid van de zintuigcellen ook terug te vinden is in de landingsreactie.

Nawoord.

Allereerst wil ik mijn promotor, Prof. Dr. J.W. Kuiper, bedanken voor zijn stimulerende ondersteuning, tolerantie en geduld. Daarnaast heeft de brede blik van de van de referent, Dr. D.G. Stavenga, voor mij, een stimulans en een leerschool betekend. Doekele, bedankt. Mijn onderzoek heeft plaatsgevonden in het Laboratorium voor Algemene Natuurkunde, waar in de Biofysica-groep uniek zintuig- en zenuwonderzoek wordt verricht. Vele medewerkers en medewerksters van dit laboratorium hebben, direct en indirect, aan mijn ondezoek bijgedragen. Ik wil ze daarvoor, bij deze, allemaal bedanken. In het byzonder moeten echter B.A. Pijpker en E. Nienhuis worden vermeld wegens hun bijdrage op electronisch en mechanisch gebied.